PRE-CLINICAL STUDY OF HERBO MINERAL FORMULATION

"*RASA KARPOORA KULIGAI*"

FOR ITS

ANTI-CANCER, ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

Dissertation Submitted to

THE TAMILNADU DR. MGR MEDICAL UNIVERSITY

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In partial fulfilment of the requirements

For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II-GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

GOVERNMENT SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI-627002

OCTOBER – 2016

GOVT. SIDDHA MEDICAL COLLEGE,

PALAYAMKOTTAI

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"Pre-clinical study of herbo mineral formula 'Rasa karpoora kuligai' for its anti-cancer , anti-oxidant and anti-inflammatory activities"** is a bonafide and genuine research work carried out by me under the guidance of **Dr.S.Kingsly M.D(S), reader,** Post Graduate Department of *Gunapadam*, Govt. Siddha Medical College, Palayamkottai, Tirunelveli-02 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

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place: Palayamkottai **Dr. S. karthik raja**

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Pre-clinical study of herbo mineral formulation *Rasa karpoora kuligai* for its anti-cancer, anti-oxidant and anti inflammatory activities"** is submitted to the Tamilnadu Dr.M.G.R.Medical University,Chennai-32 in partial fulfilment of the requirements for the award of degree of M.D (Siddha) is the bonafide and genuine research work done by **Dr.S.karthik raja** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Associateship, Fellowship or other similar title.

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"Pre-clinical study of herbo mineral formulation *Rasa karpoora kuligai* for its anti-cancer, anti-oxidant anti inflammatory activities"**is a bonafide work done by **Dr.S.karthik raja,** a candidate of Government Siddha Medical College, Palayamkottai, Tirunelveli-02 in partial fulfilment of the University rules and regulations for award of M.D (siddha) - Gunapadam under my guidance and supervision during the academic year of 2016.

Name & Signature of the Head of the department

Name and signature of the Principal

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7.0 ABBREVIATIONS

No.	Number
Mg	Milligram
Kg	Kilogram
LD_{50}	Lethal Dose $_{50}$
p.o	peros
ML	Milliliter
%	percentage
R&D	Research and Development
EDTA	Ethylene Diamine Tetra Acetic Acid
M	Male
g%	Gram percentage
g	Gram
NOAEL	No-Observed-Adverse-Effect-Level
MLD	Minimum Lethal Dose
MTD	Maximum Tolerated Dose
OECD	Organisation of Economic Co-operation and Development
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments On Animals
RKK	Rasa karpoora kuligai

ACHNOWLEDGMENT

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KMCH COLLEGE OF PHARMACY – COIMBATORE

IAEC - CERTIFICATE

This is to certificate that the project title PRECLINICAL STUDY OF SIDDHA DRUG I RAJAKARPOORA KULIGAI FOR ITS ANTI-CANCER, ANTI INFLAMMATORY, ANTI OXIDANT ACTIVITIES.

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This is to certify that Dr. KARTHIK RAJA . S

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(A UNIVERSITY U/S 3 OF THE UGC ACT, 1956)

Certificate of Participation

This is to certify that Dr. **S.KARTHIK RAJA** of **Government Siddha Medical College, Palayamkottai** participated in Ministry of AYUSH supported training programme on **"Characterization Techniques in the Standardization of Ayurvedha & Siddha Herbo-Metallic Preparations"** held during 28 to 30 march 2016.

SHANMUGHA ARTS, SCIENCE, TECHNOLOGY & RESEARCH ACADEMY

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F.No.GSMC/5676/P&D/Res/IEC/2014 Date: 16.07.2015

CERTIFICATE OF APPROVAL

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Dissertation Topic	PRE CLINICAL EVALUATION OF SIDDHA FORMULATION '***RASA KARPOORA KULIGAI***' FOR ITS ANTI CANCER, ANTI OXIDANT AND ANTI INFLAMMATORY ACITIVITY
Documents Filed	1) Protocol
Clinical / Non Clinical Trial Protocol	Non Clinical Trial Protocol
Informed Consent Document	NA
Any other Documents	NA
Date of IEC Approval & its Number	GSMC-II-IEC/2015-Br.-II/03/16.07.2015

We approve the trial to be conducted in its presented form.

The Institutional Ethical Committee expects to be informed about the process report to be submitted to the IEC atleast annually of the study, any changes in the protocol and submission of final report.

16/7/2015

Chairman
(Prof. Dr. M. Logamanian)

16/7/15

Member Secretary
(Prof. Dr. S. Soundararajan)

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This certificate is awarded to

Dr./~~Mr.~~/~~Ms.~~ S. KARTHIK RAJA

for participating as ~~Resource Person~~ / Delegate ✓ in the Fifteenth Workshop on

"Research Methodology & Biostatistics"

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 23.06.2014 to 27.06.2014.

Dr. N. KABILAN M.D. (Siddha) Reader, Dept. of Siddha	**Dr. JHANSI CHARLES,** M.D. Registrar	**Prof. Dr. D. SHANTHARAM,** M.D., D.Diab., Vice-Chancellor

AUTHENTICATION CERTIFICATE

Certified that the mineral submitted for identification by Dr.S.Karthik raja, P.G, Department of Gunapadam, Govt. Siddha Medical College, Palayamkottai are identified as

1. Rasa Karpooram (Calomel-Mercuric subchloride)

Date: 08.07.15
Place: Palayamkottai.

8/7/15

Dr. A. Kinglsy MD(s)
Lecturer
Department of PG Gunapadam
Govt. Siddha Medical College
Palayamkottai

GOVERNMENT SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI

Certificate of Botanical Authenticity

Certified the following plant drugs used in Siddha formulation **RASA KARPOORA KULIGAI** for the management of **CERVICAL CANCER (yoni putru)** taken up for Post Graduation Dissertation Studies by **Dr.S.KARTHIK RAJA (Reg No.321312003)** PG Dept, of gunapadam are correctly identified and autherticated through Visual inspection / Organoleptic Characters / Experience, Education & Training Morphology Microscopical and Taxonomical methods.

DRUG: **RASA KARPOORA KULIGAI**

INGREDIENTS:

S.N	Name	Botanical Name	Family	Parts used
1.	Betel leaf	*Piper betle*	Piperaceae	Leaves
2.	Pepper	*Piper nigrum*	Piperaceae	Dried fruit
3.	Garlic	*Allium sativum*	Liliaceae	Bulb

08.07.15

Station: Palayamkottai

Date: 08.07.15

Dr. S. SULFIN NIHAR MD(s)

Asst. Lecturer

Department of Gunapadam

Govt. Siddha Medical College

Palayamkottai

Fig : 1

allium sativum

Rasa Karpooram Purified

piper betle

Breast Milks

piper nigrum

GOVT. SIDDHA MEDICAL COLLEGE

PALAYAMKOTTAI

SCREENING COMMITTEE

Candidate Reg. No: 32121.2003

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This is to certify that the dissertation topic RASA KARPOORA KULIGAI & its Anti cancer, Anti oxidant and Anti inflammatory activity has been approved by the screening committee.

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4	Kuzhanthai Maruthuvam	Dr.D.K.Soundararajan MD(S).,	
5	Noi Nadal	Dr.S.K.Sasi MD(S).,	
6	Naju Nool Maruthuvam	Dr.M.Thiruthani MD(S).,	

Remarks:

1. INTRODUCTION

Siddha System of Medicine is belived to be originated from lord shiva the supreme god of Tamil and also he is considered to be the principal siddhar. Lord shiva preached this grateful science to shakthi, the goddess and the nanthi from them the siddha system was made available to common people by siddhars.

Siddhars where those who were not only a physican but also a social reformers. They were well versed in the filed of medicine, natural science, alchemy, astrology etc., They give many excellent medicine which cure many non cureable diseases like diabetes, TB, HIV & Cancer. Siddhars were the persons who attained siddhi, that perfection and who had overcome death through these siddha Medicines.

The truth is that throughout life we seem to swim in a sea of carcinogens and it is more by good fortune than by good management that some of us escape to die from other causes.

- William boyd

As rights said in this modern world the air we breathe, the food we eat and our life styles are all carcinogenic and are ultimately leading us by one way or other to dreadful disease cancer.Everyone in this world rich or poor, men or woman, young or old, and even animals or prone to affect by this disease and that could be prophylatically given against this disease. Also there is no medicine in the world that good completely cure this disease.

The Currently available treatments for Cancer viz Radiation, chemotherapy, Surgery are all of very Limited Value. The Surgical Procedures are useful only is very early stage and it is miserable that the disease is often detected in advanced stage. The Radiation technique and chemotherapy are useful only to prolong the survival period.

In the pathetic Situation when scientist all over the world struggling to formulate a new medicine to combat the disease. In our Siddha system of medicine we had thousands of medicine with the indications to cure the disease.

I selected yoniputru (cervix Cancer) as my dissertation topic because it is the most frequent of all genital tract cancer in women.

Among various medicine in Siddha literature. I selected Rasa karpoora Kuligai as my dissertation drug. Which Contains Mercurial Compounds (Pooram) that are used to cure almost all the diseases in Siddha.

We should be very careful while using Parpam, Chendooram, Kattu, Kalanugu, Chunnam, Guru, Kuligai etc. because these are prepared from metals, poisonous metalic salts. So, preparation of this type of internal medicine required high skilled work called suddhi. It is the important procedure which is meant purification of the raw drugs; it is the first step in medicine preparation.

If the prepare medicine by using raw materials without suddhi It will produce immediate dangerous ill effects are delayed toxicity or both. So siddhars have concentrated very much in suddhi and recommended to many methods for each drug to purify them.

Eventhough we are prepared the drugs after purification some times they produce some adverse effects. It may be due to improper purification, preparation or dosage.

So majority of drugs prepared from mercurial substances are toxic unless other wise they are prove as safe by universely accepted toxicological screening methods. So it is essential to study about a drug regarding at what extend it is safe.

As we are having advanced medical technology now a days there is a long gap between recent findings and traditional belives. It is time for us to establish our siddha system by breaking this interruption. So it is our duty to introduce the drug standardisation in universely acceptable method.

A study on cancer is a life long work and with limited facilites and limited time this dissertation work is only a preliminary study, when compared to those well equipped brilliant team of researchers and scientist all over the world spending crores of money and Conducting life long trails. This work of mine is simply a drop ina mighty ocean. But I have tried my best with all sincerity and dedication to study the action of the drug on the disease.

By keeping all these facts in mine I enter in to this dissertation.

2.AIM& OBJECTIVES OF THE STUDY

AIM:

The aim of this present study is to scientifically validate traditional siddha medicine*Rasa karpoora kuligai*for its Anti-cancer, Anti-oxidant, Anti-inflammatory activities and also access safety profile of these drug according to OECD guidelines.

OBJECTIVES:

- The prime objective of these studies to collect various literatures which includes traditional siddha literatures basic modern science aspect of the test drug and the diseased view.
- To prepare the drug according to siddha classical literature illustrations to standardize the drug by appropriate physico-chemical analysis.
- To analyse the drug chemically for reduction of metals, minerals to access the safety profile of the drug by acute and sub-acute oral toxicity profiles of *Rasa karpoora kuligai* according to OECD guidelines.
- To evaluate the anti-cancer activity of the test drug *Rasa karpoora kuligai* by invitro HeLa cell line method.
- To access anti-oxidant activities of the test drug *Rasa karpoora kuligai* by using invitro dpph radical scavenging method.
- To enumerate the anti-inflammatory activity of test drug *Rasa karpoora kuligai* by using carrageenan-induced paw edema method.
- To evaluate exact bio-chemical content of *Rasa karpoora kuligai* by using various standard protocol.
- To evaluate the mineral content of *Rasa karpoora kuligai* by various instrumental analysis.
- To evaluate the efficacy of the drug *Rasa karpoora kuligai* by various analysis.

3. REVIEW OF THE LITERATURE

3.1. RASA KARPOORAM (இரச கற்பூரம்)

3.1.1 GUNAPADAM ASPECT

Rasakarpooram does not find a place in the list of 64 padanas but is considered as one among them by the medical practitioners. It's prepared by the combination of rasam and salt.

SYNONYMS:

"துர்க்கைகளையெலியிடைச்சனிபூரந்
தீட்டுவிடதாலிநக்கிபூடிமதாந்"

- நாமதீபநிகண்டு 29

- Durgai
- Kalai
- Aellidai
- Sani
- Pooram
- Thettu
- Vidathaali
- Nacki
- Buddimaatham

RASA KARPOORA VAIPU MURAI: (Method of preparation)

FIRST METHOD:

"தானென்றகற்பூரமொன்றுசொல்வேன்
சாதகமாய்சூதம்ரெண்டுதூக்கி
வானென்றசட்டிக்குள்மூன்றுபடியுப்பை
வளமாகபொடித்திட்டுநடுவேகேளு
தேனென்றசெங்கல்தூள்கால்படிதானிட்டு
திரமாகக்குளித்ததிலேசூதம்விட்டு
ஏனென்றமறுசட்டிகவிழ்த்துமூடி
இயல்பாகவெழுசீலைமண்ணும்செய்யே
மண்செய்துதொண்ணூறுகடிகையப்பா

வாகாகமூத்தீயுமெரித்துமைந்தா

மண்செய்தமேல்சட்டிக்குள்ளேகேளு

வாகாகஉரைத்திருக்கும்வெள்ளைமெதத்

மண்செய்தநற்பூரங்குழாயில்வைத்து

வாகாகபணவிடைதான்தூக்கிக்கொண்டு"

- **Agasthiyar paripulanam 400.**

2 palam sootham placed in the mud pot in between 3 padi culinary salt(Nacl) and brick stone powder.Close the pot with another mud pot and seal with 7 mud seelai . It is burnt for 90 kadikai after it is colled pooram is found deposited on the upper pot and the same is collected.

SECOND METHOD:

மயக்கமுறுமிரதகற்பூரஞ்சொல்வோம்

வியாதியெல்லாந்தீர்ந்துபோம்வரிசைகேளு

மயக்கமுறஉப்புளுக்குச்செங்கல்தூளும்

வரிசைபெறவழித்தெடுத்துவைத்திடாயே

வைத்ததோர்இருவகையுந்தூளாயாட்டி

மைந்தனேசட்டிக்குள்பாதிநீயும்

வைத்திடுவாய்அதிற்குகையைச்சூதமப்பா

வாராடாகழஞ்சுபத்துமாற்றமன்றி

வைத்தங்கேகுகைவாயில்பொடியுமிட்டு

மறுசட்டிகொண்டிட்டுவாயைமூடி

வைத்துநீயேழுசீலைமண்ணும்பூசி

மாயிபதம்பூசித்துஅடுப்பிலேற்றி

அடுப்பேற்றிகமலவன்னிதினந்தானொன்று

அதன்பிறகுகடாக்கினியுந்தான்மூன்று

எரித்தாறியெடுத்துப்பார்சட்டிமேலே

ஏறிநிற்கும்கற்பூரம்எடுத்துவைத்து

10 kalanjuof sootham placed it mud pot in between the well powdered culinary salt and brickstone powder close the mud pot with 7mud seelai.then burnt it as deepackini kamalackini and kaadackini for 3 days respectively after it is cooled the pooram is found deposited on the upper pot and and the same is collected.

THIRD METHOD:

Sulfur 67.2gm is melted in mud pot and mercuery 336gm is added to it and kinded well and there forms ablack colourd powder (kajali). Brickstone powder is placed up to half of the level of a pot culinary salt (Nacl) 650 gm is placed over it . Mercury sulfer camphor is placed over the salt and sealed with mud paste cloth . It burnt for 12 hour with kadackini after it is colled the mercurous chloride is found deposited on the upper pot and the same is collected.

PROPERTIES:

Colour	-	White
Appearance	-	Heavywhite rhombic crystals
Potency	-	Hot
Taste	-	Salty, Pungent

ACTION:

- Laxative
- Tonic
- Antiseptic
- Diuretic

It is also an after excessive bile producer.

THERAPEUTICAL EFFECTS:

“இடைவாதசூலையெரிசூலைகுன்மந்
தொடைவாழைவாதமாஞ்சோணி - யிடையாதோ
வொக்குரசகர்ப்பூரமொன்றேயனவொருதல்
இக்குவெல்லத்தேழுநாளீ”.

“சசிவன்னகருப்பூரத்தில்
சாதித்தகயஞ்சுவாசம்
பசிகலிதாபசோபம்
பவுத்திரம்பிளவைகுஷ்டம்
வசிதருகிராணியோடு
வளரதிசாரமேகம்
இசிதருமிசிவுசூலை
யிவைபலரோகம்போமே”.

“திரண்டவாதங்குடல்வாதம்

தீருஞ்சந்நிபதின்மூன்று

மருண்டேகுத்துமரையாப்பு

மண்டைச்சூலைகபாலவிடி

பரங்கிச்சூலைபற்கிரந்தி

பக்கசூலையிவைமுதல்போம்

இருண்டமேனிபொன்னிறமாம்

இதுவேகற்பம்இயம்பீரே”.

When calomel is taken along with jaggery for seven days,

- It cures various types of throbbling pains,
- Throbbling pain in the lumbar region,
- Burning sensation,
- Ulcer due to disorders of vatha humours,
- Hepatomegaly,
- Pyrexia,
- Jaundice,
- Basillary dysentery,
- Dropsy,
- Chronic ulcer,
- Venereal diseases,
- Indigestion,
- Vomiting,
- Diarrhoea,
- Worm infestation,
- Rheumatism,
- Itching,
- Constipation,
- Scabies,etc.,

It is also effective in the treatment of head ache as explaind in the above tamil verses. In siddha system of medicine his compare to sanjeevi because it cures hard disease

like syphilis(kiranthi), uterine cancer(allgul putru), uterine ulcer(allgul ranam) and chronic non-healing ulcers.

- AVN 4th vol

PURIFICATION OF CALOMEL:

FIRST METHOD:

The poultice made of betel leaf(piper betel) and pepper (piper nigram) each 8.75gm,is taken and dissolved in 1.3 litre of water calomel 35gm and tied with a cloth and immersed in the liquid from the crops bar and heated. After the water is reduced to ¾ of its volume the calomel is taken out washed with water and dried to get it in purified form.

SECOND METHOD:

Calomel 35 gm is consolidified in mother's milk for 3 hours and again it is consolidified in garlic oil(thailam) for 9 hours. It is taken out as purified.

THIRD METHOD:

Before mixing calomel in electuraries, it is consolidified with the juice of mukia madraspatana (musumusukku) and washed

OTHER CANCER MEDICINES WHICH CONTAIN RASA KARPOORAM AS CHIEF INGREDIENT:

1. CHANDA MAARUTHA CHENDOORAM**:**

Dosage	:	½ - 1 kundri
Adjuvent	:	palm jaggary, tripala legium, panjadepakkini legium.
Indication	:	Aruvagai putru (six types of cancer) Pavuthiram (fistula) Paarisavaayu (hemiplegia) Mugavadham (facial palsy)

2. VAAYU MAATHIRAI**:**

Dosage	:	½-1 maatirai
Adjuvent	:	Palm jaggary, sukku paste.
Indication	:	Uterine cancer (Allgul putru) Penial cancer (Linga putru) Necrotic ulcer (Ali ranam)

3. CHITHIRAMOOLA KULIGAI**:**

Dosage	:	1 Melagu
Adjuvent	:	Palm jiggery
Indication	:	Uterine cancer (Yoni putru),
		Penile cancer (Linga putru),
		8 types of ulcers (Gunmam).

4. KOOROSANAI MELEGU**:**

Dosage	:	3-5 kundri
Adjuvantent	:	Sugar, palm jiggery
Indication	:	Uterine cancer (allkul putru)
		Syphilis (kiranthi)
		Amenorrhoea (soodaka kattu)

5. POORA KATTU

Dosage	:	1-4 rice weight
Adjuvantent	:	parangipattai legium, vaalmilaggu legium.
Indication	:	Uterine cancer (allgul putru)
		Syphilis (kiranthi nooi)
		Urinary bladder ulcer (neerpai ranam)

6. VETTAI MELEGU**:**

Dosage	:	1/2 – 1 kundri
Adjuvantent	:	palm jiggery, butter
Indication	:	vettai (venelial diseases)
		Aan kurri pan (penear ulcer)
		Neerthaarai pun (ureter ulcer)

7. POORA CHUNNAM
8. CHITTATHE ENNAI
9. NAVA KIRAGA ENNAI
10. VACHIRA KANDI MATUIRAI
11. SHANMUGA CHENDOORAM
12. PANJASOOTHA MELEGU
13. KARPOORA MATHIRAI
14. OONAN SUDAR THAILAM
15. NAVA PAASAANA THILAM

SIGNS AND SYMPTOMS OF CALOMEL POISONING:

Multiple red boils may appear on the face, ache formation, ulcers in the chest, mouth and tongue diarrhoea and dysentery, scrotal swelling and ulcer in the uvula.

ANTIDOTE FOR POISONING:

Nilapanai kilangu (curculigo orchides)	-	8.75gm
Vallarai ver (centella asiatica root)	-	8.75gm
Ponnankanni ver (alternatheria sessiles)	-	8.75gm
Kanduparangi ver (clerodendrumserratum)	-	8.75gm

All the above ingredients are mixed and boiled to make a decoction. This decoction is used twice a day for two or three weeks with suitable diet restrictions.

3.1.2 GEOLOGICAL ASPECT

MERCURY SUBCHLORIDE:

Mercury sub chloride is a chemical compound with the formula Hg_2cl_2. Also known as calomel (a mineral form rarely found in nature)

IUPAC name	:	Dimercury dichloride
Other name	:	Mercurous chloride
		Calomel
		Horn mercury
		Mercury (I) chloride
Chemical formula	:	Hg_2cl_2
Molar mass	:	472.09 g/mol
Appearance	:	Heavywhite rhombic crystals
Density	:	7.150 g/cm^3
Melting point	:	525°c
Boiling point	:	383 °c
Solubility in water	:	0.2 mg/100ml
Solubility	:	Insoluble in ethanol, ether
Refractive index	:	1.973
LD_{50} median dose	:	210 mg/kg (rat, oral)

OCCURRENCE:

- Haria,
- Obermoscuel,
- Herowithz in Bavia and
- Armaden in spain

PREPARATION OF MERCUROUS CHLORIDE:

It is the most important mercurous compound. In nature it occur as horn quick silver. It is prepared by

1. $HgSo_4 + 2Nacl + Hg \rightarrow Hg_2cl_2 + Na_2So_4$

Subliming a mixture of mercuric sulphate, mercury and sodium chloride in iron pot

2. $Hgcl_2 + Hg \xrightarrow{\Delta} Hg_2cl_2$

By heating an intimate mixture of Hg_2cl_2 and Hg in appropriate mixture proportions in iron pot.

3. $Hg_2(NO_3) + Nacl \rightarrow Hg_2cl_2 + 2\,NaNo_3$

$Hg_2(NO_3) + 2Hcl \rightarrow Hg_2cl_2 + 2HNO_3$

By adding a soluble chloride, Eg : Nacl or dill Hcl to the solution of $Hg(NO_3)$.

4. $2Hgcl_2 + 2H_2O + SO_2 \rightarrow Hg_2cl_2 + 2Hcl + 2H_2SO_4$

By reducing $Hgcl_2$ solution with SO_2

ACTION:

- Cathartic
- Alterative
- Diuretic
- Antiseptic
- Anthelmentic.

INTERNAL USES:

- Constipation,
- Cholera,
- Dysentery,
- Cardiacdropsy,
- Pleurisy,
- Malign fever
- Malaria,
- Syphilis,
- Gout,
- Worms,
- Cholelithiasis,

- Mitral insufficiency,
- Eclampsia,
- Gravidorum.

EXTERNAL USES:

- Small pox pitting,
- Pruritus,
- Diphtheria,
- Syphilitic ulcer,
- Myiasis,
- Membraue croap,
- Condylomata warts.

3.1.3 LATERAL RESEARCH

Calomel was a mercurial compound used extencively by both union confederate doctors to treat wide variety of medical conditions. It came in two main forums. “Blue Pills” contained a mixture of mercury rose wate, licorice, powedered rose, honey and sugar. “Blue Mass” was a lump of Mercurous chloride from which dispensing doctors pinched of a place. **Doses were never standard**.

In 19th centaury doctors feared constipation and dispensing calomel was one of the main methods of keeping the bowl open. However, strangely enough, calomel was also given to treat diarrhoea and dysentery. Many physicians seemed unaware that the doses of calomel and a related compound, tartar emetic, often caused worse problems that the original conditions.

The **large and frequent doses of mercury** compounds cause excessive salivation often a pint to a quart a day. Many patients receiving these “heroic” doses, suffered from mercurial gangrene death of cheek and mouth tissue that often led to permanent facial deformities. Lose and lost teeth were common, as was death from mercury poisoning.

In 16th centaury paracelsian and china physicians are used to treat malaria and yellow fever and a preparation called “worm chocolate” and “worm candy” was given to patients infested with helminthis.

Toxic effects were soon noticed in individuals given **large doses for long periods**, in which excessive salivation, gum inflammation, loosening of teeth, gastro-intestinal upset and an ashen appearance developed. They had troubling neurological symptom, such as arm and facial tremors, hyperfine, ataxia and erethism, unusual timidity and personality change.

The toxic potential of calomel was highlighted in 1948. A Cincinnati paediatrician discovered that a common infantile and childhood illness called “Aerodynia” or “Pink disease” was caused by the white spread use of calomel in treating childhood teething and constipation. As late as 1950 acrodynia accounted for more than 3% of admissions to children ward’s in London hospitals. Official statistics record that 585 children died of pink disease between 1939 and 1948 in England and waves.

“Effect of dose not in proportion to size” well – triturated doses better than large coarse one.

3.2 ALLIUM SATIVUM –வெள்ளைப்பூண்டு

3.2.1 GUNAPADAM ASPECT

VELLAIPUNDU SYNONYMS:

வெள்ளுள்ளி தன்னுடைய விவரங்கேளு

மேதினிக்குறை சோனா லசு நோலிஷ்டோம்

மள்ளுள்ளி மதுசிய கெந்தாங் கேள் அஸ்தமாம்

மகாகந்தோய்கோ நேர் நியாகு கிரஞ்சனோ

மள்ளுள்ளி தீபத்திரக்கமாகுஞ்

செப்பியதோர் வெள்ளுள்ளிச் செயலுமாமே.

SYNONYMS:

- Kuraisonalasu
- Nolistoo
- Madhusiya kaentharam
- Kael astham
- Maha kanthoo
- Koonaerniyaa
- Kiranchunoovaa
- Depathirackam
- Lasunam
- Kaayam
- Ulli
- Pundu
- Vellai pundu
- Velvengayam

VERNACULAR NAME

Eng	:	garlic
Tel	:	thella, gadda , velulli
Mar	:	lohson
Mal	:	vellulli
Kan	:	bellulli
Sans	:	lasuna
Hind	:	lashan

HABITATE

It s cultivated all over india. It s more pungent than onion nellakiri pundu is more popular.

PARTS USED:

Bulb and oil.

ORGANOLEPTIC CHARACTERS:

Colour	-	white
Odour	-	Garlic Odour
Taste	-	pungent (karppu)
Character	-	Veppam
Pirivu	-	Pungent

- *Gunapadam Mooligai Vaguppu*

CHARACTERS:

Taste - pungent,

Potence Character-Hot potency

Biotransformation-pungent [3]

GENERAL CHARACTERS:

சந்தியோடு வாதந்தலைநோவு தாளின்வலி
மன்னிவரு நீர்கோவை வன்சீத – மன்னமே
யூள்ளுள்ளிக் காண்பா யுளமூல ரோகமும்போம்
வெள்ளுள்ளிக் தன்னால் வெருண்டு

- Garlic used for deafness chronic cough, asthma dysentery, headache, cyanicites, haemmoroids.
- It also used for vadha diseases, kapha headache and oral diseases.
- Garlic taken along with pepper and karichallai for stomach problems
- 1 to 2 drops of garlic juice droped into ear for ear diseases and deafness
- garlic juice processed along with gingelly oil and used for ear diseases
- 20 to30 drops of garlic juice used 2 to 3 times per a day for chronic cough, asthma.
- Garlic juice processed with mustard or coconut oil then it is externally used for body pain, pricking pain, whezzing, vadha diseases.

ACTIONS:

- Carminative,
- Stimulant,
- Expectorant,
- Anthelminthic,
- Anti oxidant,
- Stomachic,
- Tonic,
- Alterative,
- Diuretic.

AYURVEDIC AND SIDDHA USES:

- Clove of garlic was known as ahome remedy in olden days in the east and is one of the most useful on account of it s prophylatic and curative properties.
- The garlic oil capsules product the human body from the attacks of bacteria and bacillae in times of epidemics or when the danger of infection is prevalent and containing all the curative properties.
- These capsules renew the blood cleanse if of all impurities regulate the digestion and remove all parasites in the intestines which might be injurious to health
- The capsules recommented fof diseases of the lungs arterio-sclerosis high blood pressure gout rheumatism asthma chronic bronchial catarrh intestinal complaints loss of appetite constipation and worms.
- The oil from seeds is prescribed internally as a febrifuge to prevent recurrence of the cold fits of intermittent fever
- Externally it s used in paralytic and rheumatic affections as resolvent the garlic is applied to indolent tumours.
- Internally garlic given with common saltin affection of the nervous system headache flatulence hysteria cough etc.
- In emergency conditions it s applied like onion to the nose in cases of fainting
- Externally the juice used as a rubefacient liniment acts very beneficially in infantile convulsions other nervous and spasmodic affections relax sorethroat inasthma general paralysis facial paralysis gout sciatica
- It s used for skin diseases including leprosy bruised garlic and onions are applied to the chest as poultice
- When eaten in cold season it is said to ward off attacks of rheumatism and neuralgia

- Garlic produce copious dieresis and therefore it is used in dropsy and anasarca
- Garlic juice mixed with 3-4parts ofordinary or distilled water has been used as a lotion for washing wounds and foul ulcers
- Definite improvement in the condition of infected wounds was noticed with in 24 hours after washing with this lotion and a very marked and decided improvement with in 48 hours not only was the prulent discharge markedly decreased but pain also considerably relived and in some cases in entirely disappeared no injury to the tissues could be noticed as a result of application of this solution.

OTHER SIDDHA FORMULATIONS:

1. KAALAMEGA NARAYANA CHENDOORAM:
   Dosage : 30-100mg
   Adjuvent : Thippili powder
   Indications : Uterine cancer, Cheek cancer
2. THAMIRA CHENDOORAM:
   Dosage : 30-45mg
   Adjuvent : Garlic juice
   Indications : Weakness
3. PANCHA SOODHA MELUGU:
   Dosage : 50-100mg
   Adjuvent : Garlic juice
   Indications : Vadha diseases, five types of pain
4. PASAANA PARPAM:
   Dosage : 1 milagalavu
   Adjuvent : Garlic juice
   Indications : Many diseases
5. KANTHAGA MELUGU:
   Dosage : 2-3 Kundri
   Adjuvent : Garlic juice
   Indications : Leprosy, Diabetes insipidus

3.2.2 BOTANICAL ASPECTS:

ALLIUM SATIVUM

Taxonomy Classification

Kingdom : Plantae

Division : Angiosperms

Class : Monococs

Order : Asparagales

Family : Amaryllidaecae

Subfamily : Allioideae

Genus : Allium

Species : A.sativum

Vernacular name

Eng : allium sativum

Sans : Lacuna, Ugragandua, Bhutagua, Mahusudra, Rosanam

Hind&Bom : Lasan

Sind : Thum

Per : Sir

Gug : Lasan, Shutam

Mah : Lasan

Tel : Vellulli, Tellagadda

Tam : Vellapundu, Vallai Pundu, Ulli Pundu

Mal : Vellulli

Can : Bellulli

Ben : Rasan

Parts used:

Bulb and oil

Distribution:

Garlic is among the oldest knowing horticultural crop. In the old world Egyptian and Indian cultures refored to garlic 1000 years ago and there is clear historical evidence for it use by the Barsilonians 4500 years ago.

Garlic grows wild only in Central Asia (Centered in Kgrgyzstan) today. Earlier in history garlic grew wild over a much larger region and, in fact, wild garlic may have occurred in an area from china to India to Egypt to the Ukraiue. It's cultivated all over the world in Spain, France, Egypt, Bulgaria, Hungary, USA, Mexico and Brazil.

Botany:

Garlic is perennial of the lily family. It grows to a height of about 60 cm. It has short, flat upright leaves 15-30cm. The tall single flower stem bears spherical head of pale pinic or greenish- white blooms often mixed with tiny bulbis the subterranean white-skinned bulb or corn is subdivided into numerous "cloves".

Action:

- Hot,
- Stimulant,
- Carminative,
- Emmenagogue,
- Antirheumatic,
- Anthelmentic,
- Alterative.

Organoleptic properties:

The bulbs are pinkish-white color and are odoriferous. The size of the bulb varies in between 1.5-2.5 cm.

Morphology \Macroscopical Characters:

- The bulb grows at the base of a perennial plant with a erect flowering stem that grows 2-3 ff long.
- The bulb is made up of several outer thin protective sheaths covering the inner sheathes. The inner sheath covers the swollen leaves called as cloves. The mature bulb has around or more cloves in each bulb.
- The cloves gave no symmetry except for a few present in the center.

Microscopic characters:

The bulbs are covered by an outer scale. The outers scale is made up of an epidermis which encloses a mesophyll (devoid of chlorophyll), a ground tissue and below it is a layer of lower epidermal cells.

The dry scales also contains about 2-3 layers of rectangular cells.

The rectangular cells may have many rhomboidal crystals of calcium oxalate

The epidermal cells contain parenchymatous cells connected to several rectangular cells and vascular bundles made up of alternating xylem and phloem.

The epidermal cells contain thick pitted walls.

The lower epidermis consists of smaller rectangular cubical cells.

Phytochemistry:

Garlic bulbs are made up of numerous minerals, vitamins, carbohydrate, amino acids, volatile oils and other trace elements.

Amongest all the members of the Allium species, garlic is said to have to the highest sulphur content.

Volatile oils are present in about 0.1-0.5% concentration in garlic. These constitute of sulphur containing compounds like diallylsulphide, diallyltrisulphide, methylallyl sulphide, allyl propyl disulphide, allin, ajoene etc.

When the garlic clove is crushed allin (s-allyl-1-cysteine sulpoxide) by the action of the enzyme allinase gets converted to 2-propene-2-sulfenic acid which in turn dimerizes to allicin (diallyl thio sulfinate)

Allicin is responsible for the pungent odour of crushed garlic and also for zone of its pharmacological activities of garlic.

Vitamins like vitB, VitA, VitC etc, 17 amino acids including 8 essential amino acids and minerals like Phosphorus, Calcium, Magnesium, Pottasium, Iron, Selenium, Germanium etc are present.

Garlic medicinal uses

In Hypercholesterolemia– Garlic has said to lower cholesterol levels. The proposed mechanism for this is that the **diallyl disulphides** and **diallyl trisulphides**present in garlic oil interfere with the factors normally responsible for lipid synthesis. Garlic can reduce the activity of the thiol group in the enzymes in the body. Garlic oil can also carry out oxidation of NADPH. Thus both the above activites interfere with normal lipid synthesis and blood lipid levels are reduced. These thiol containing enzymes are HMG-CoA reductase and coenzyme A which are essential enzymes for cholesterol biosynthesis.

Other proposed mechanisms for reducing lipid levels are increased loss of bile salts in the feces. It has also been suggested that mobilization of these lipids into circulation can also reduce lipid levels.

As Antithrombotic agent– **Ajoene** (4, 5, 9-trithiadodeca-1, 6, 11-triene-9-oxide) present in garlic is considered to inhibit platelet aggregation and is the most potent antithrombotic component of garlic.**Methylallyltrisulphide** present also acts as an antithrombotic agent. The suggested mechanism for this is the interference with thromboxane synthesis.

It also shows fibrinolytic properties thereby helping in clot degradation.

Antimicrobial properties– Garlic shows antimicrobial activity against various pathogens such as bacteria including resistant types, fungi, virus etc. It is active against both gram positive and gram negative bacteria and strains of Mycobacterium. These effects are seen due to **ajoene** present in garlic. **Allicin** also shows the antimicrobial properties by inhibiting thiol containing enzymes thereby affecting protein, DNA, RNA synthesis.

Due to the antifungal properties, garlic has been proposed for the treatment of oral and vaginal candidiasis.

Garlic is active against various viruses such as herpes simplex virus type I and II, Infulenza B virus, parainfluenza virus, cytomegalovirus, human rhinovirus type 2 etc.

Chemoprotective properties– Animal studies have shown that garlic has positive effects against hepatoxins. The reaction of **allicin** in garlic with the sulfhydryl groups contribute to the inhibitory effect. Sulfhydryl groups concentration is high in rapidly dividing cells.

Immunity– Immunity is increased due to a number of factors on consumption of garlic. **Selenium** and**germanium** present in garlic are said to be responsible for immunologic activities. Enhanced phagocytosis, increased killer cell activity, lymphocyte proliferation, increased production of cytokines and reduction of immune suppresion are the suggested mechanisms via which garlic increases immunity.

Antioxidant properties– **Allicin** present in garlic is responsible for the increase of catalase and glutathione peroxidase enzymes which are two important antioxidant enzymes in the body. The other sulphur compounds may also show potential antioxidant properties by inhibiting lipid peroxidation in the liver and preventing a reaction which is considered to be one of the main features of aging in liver cells.

3.2.3 LATERAL RESEARCH

Anti-carcinogenic activity:

- Garlic also helps to reduce the risk of cancer due to its carcinogenic properties. It helps to prevent cancerous compounds from forming and developing into tumours. It also inhibits the growth of tumours that have already formed.
- This DADS and s-allyl cysteine (SAC) decrease the incidence of colon and mammary cancer.

Anticarcinogenic and antitumerogenic

- Several epidemological studies have shown that garlic consumption is correlated to reduced cancer risk.
- Individuals consuming about 20 gm of garlic per day have been reported to show a 13 times reduced stomach cancer mortality rate then individuals consuming less than 1 gm per day according to studies conducted on population of different regions of contries like China , Italy and United States.
- In lowa women's health study the results showed the garlic is significantly associated with decreased colon cancer risk and reported to decrease the risk of gastric cancer.
- Animal and in vitro studies have demonstrated that various garlic constituents inhibit the growth of different forms of cancer cells such as skin, mouth, mammary, lung, uterine, oesophagus,stomach and colon tumours,.
- In fact, tumours that have already formed can be reduced by 50-70% by increasing garlic intake
- Several compounds are involved in garlic possible to anticancer effects. Garlic contains allyl sulphur and other compounds that slow down and prevent the growth of tumour cells. Allyl sulfer compounds, which occur naturally in garlic and onions, make cells vulnerable to strees created by products of cell division. Because cancer cells divide very quickly, they generate more sterssors than most normal cells. Thus cancer cells are damaged by the presence of allyl sulphur compounds to much greater extent than normal cells.
- The chemistry of garlic is complicated as a result; the quality of garlic products depends on the manufacturing process. Peeling garlic and processing garlic into pill or powder can increase the number and valiety of active

compounds. Peeling garlic releases an enzyme called allinase and starts a series of chemical reaction that produce diallyl disulfide (DADS)

- In garlic powder s-allyl cysteine (SAC) (or) dialkyldisulfide (DADS) decrease the incidence of colon cancer and mammary tumours.
- SAC and s-propyl cysteine inhibit the invitro formation of N-Nitroso morphoine (NMOR) a know carcinogen.
- Garlic and onion have been reported as anticarcinogenic food due to their ability to detoxify the xenobiotics.
- Dially Disulphide (DAD) isolated from garlic is reported to inhibit the activation of Nitrosamine, than reducing the probability of the development of cancer of the stomach.
- Garlic can inhibit the progression of existing cancer. It is more effective against N-Nitroso induced cancer.
- Garlic contains number of sulphur compounds including diallylsulfide. This compound inhibits tumour metabolism and enhances the immune response.

Anti-Microbial Activity:

- Garlic has been embloyed for centuries as a traditional herbal medicine to treat infectious diseases.
- Studies with garlic extract confirmed its antiviral activity against influenza-B and herpes simplex viruses.
- Garlic juice showed Antibiotic activity against Mycobacterium tuberculosis, Staphyllococcus aureus, Brucella abortus and Escuerichia coli.
- Garlic extract was found to be capable of inhibiting 22 isolates of the fungus.

3.3. PIPER BETEL (வெற்றிலை)

3.3.1. GUNAPADAM ASPECT

VETRILLAI SYNONYMS

வெற்றிலையின் பேர்தனையே விளம்பக்கேளு
வேண்முயதோர் தாம்புல மாதாவாகும்
சித்திலையாஞ் சாதகலட்சுமி யாகும்
தாம்பூலக் சண்ணி நல நாகவல்லி
ஒத்திலைவோம் புலக்கன்னியாகும்
உறுபல்லுக் கழகிதான் தேகரக்சிகயாம்
நத்தலை நாகத்தை சூரணமாக்கி
நலங்கியதோர் வெற்றிலையின் நாமமாமே

Synonyms:

- Saathaga lakshmi
- Thamboola madha
- Nagavalli
- Lomalaga kanni
- Thaeka rakshini
- Palluckalaggi
- Nagatthai chooranamaacki
- Thamboolam
- Thamboola valli
- Thirayal
- Mellilai
- Vellilai
- Melladagu

Vernacular names:

Telugu	:	Tamalapaku
Kannada	:	Vilaya
Duk	:	Thambole
Bengali	:	Pan
Hindi	:	Paan
Sanskrit	:	Tambula and nagavalli
Gujarathi	:	Naagarvel na paan

Types:

Vetrillai	:	Migundha manamum, kaaramum, nivamum illatuattu
Kammaru vettrilai	:	Karuppu niramum, karppum udayatu
Karpoora vettrilai	:	Karpoora manamum siru kaaramumudaiyatuu

habitate:

In India it's cultivated in hot areas and saduppu nilam. It's a type of climber. It's cultivated for its leaf.

Parts used:

- Leaf

Properties:

Taste (suvai)	:	vurviruppu, pungent (karppu)
Quality (thanmai)	:	veppam (hot)
Pirivu (postdigstive effect)	:	pungent (karppu)

Action:

- Stimulant,
- carminative
- astringent,
- aphrodisiac,
- antiseptic,
- febrifuge,
- stomachic,
- galactagogue,
- sialogogue.

General properties:

ஐயம் அறுங்கான் அதன் சாரங் கொண்டக்காற்
பையச் சயத்தியம்போம் பைந்தொடியே! – மெய்யெண்
கடியின் குணம் போகும் காரவெற்றி லைக்குப்
படியுமுத் தொடமிதைப் பார்

எட்டிலொன்று கிட்டினீ ரேற்றங் சிரோபார
மாட்டி விடுசன்னி மாந்தமோடு – நாட்டிற்
பரிய குரக்கம்மல் பண்டியுப்பி சம்போ
மரியகம் மாறு வெற்றிலை

- Juice of betel leaf curesIyyam, saytthiyam, kanaakkadi, muppini, uteriaria.
- Betel leaf juice cures cynacites, head ache, stomach pain, sorethroat, muppini, maantuam vaetriuppisam.

Therapeutic uses:

- For sore throat and thondaiyaddaippu betel leaf is taken along with saambrani pathangam.
- Paarsurakkam, paalkatti udayavum betel leaf anaill vaatti adukkadukaai mulaill vaithu kattalaam.
- Tualai paluvukku muckilum, kaathu kutthalacku kaathilum vetrilai juice 2.3drops.
- Betel leaf juice give along with ginger juice for lung disorders.
- Its externally used for fire wound.
- Vetrilaiyai ennaiyil nanaithu, vilakil vatti maarbin mel poda, eramal, moochumuttal kadinaswasam, kuzhanthaigalukundaagam erumal vilagum
- Koorosani taken along with betel juice for duspnoea, cough and koolaikattu.
- Mercury purified by betel juice.
- Fresh leaves are generally used for chewing, in the form of packets made with the addition of hurnt like catecha or gambler and pieces of areca(betel) nut in any state of maturing and tobacco.
- Those who can afford, add also cardamoms, nut megs, cloves, camphor and aromatics.
- They sweeten the breath, improve the voice and remove from mouth.
- Also they increase the salivary secretion. The ancient Hindu writers' recommented betel leaves to be chewed early in the morning after meals.

Siddha and ayruvedic uses:

It is useful in bronchitis, asthma, catarrh, cough, leprosy, skin diseases, filarial dipsia, alcuolism, syncope, otalgia, fever, halitorsis impotencym rewmatism, dyspepsia, indigestion in children, pnaryngitis, laryngitis, obesity,conjutivitis, night blindness, glandular swelling oil of betel has been used in the treatment of various respiratory cattarrns and as a local application either by gargle or by ingalation in diphtheria. It has carminative properties oil shows marked irritant action on skin and mucous membrave. It has an antispasmodic action on involuntary muscle tissue, inhibiting excessive peristalitic movements of intestinals.

OTHER SIDDHA FORMULATIONS:

1. ABRAHA PARPAM:

   Dosage : 100-200mg

   Adjuvent : Honey

   Indication : Diabetes Mellitus and Diabetes Insipidus

2. POORNA CHANTHROTHAYAM:

   Dosage : 100-200mg

   Adjuvent : Betel Leaf juice or Karpoorathi chooranam

   Indication : Tuberculosis, Dysentry, Fever

3. KASTHURI MATHIRI:

   Dosage : 1-2 pills

   Adjuvent : Betel Leaf juice or Honey

   Indication : Heart proplems, intermittent fever, fits

4. SAANTHA SANTHROTHAYAM:

   Dosage : 1-2 pills

   Adjuvent : Betel Leaf juice or Honey

   Indication : Liver diseases, constipation

5. SAMBARANI POO MATHIRAI:

   Dosage : 1 pill

   Adjuvent : Betel Leaf or Pepper powder

   Indication : Dysmennorhea, Amennorhe

3.3.2 BOTANICAL ASPECT

PIPER BETEL

BOTANICAL ASPECT

Botanical name : Piper betel

Taxonomy classification:

Kingdom	:	Plantae
Division	:	Angiospermae
Class	:	Magnoliidae
Order	:	Piperals
Family	:	Piperaceae
Genus	:	Piper
Species	:	Betel
Botanical name	:	Piper betel

Vernacular name:

English	:	betel pepper
Ayurvedha	:	tambula, nagavallari nagini, tambulavalli, saptashiva,
Siddha	:	vertillai, naga valli, kanmaru vetrillai
Unani	:	pan, tambool
Telegu	:	tamala paku, tamula paku
Marathi	:	pan, nagvel vidyachepan
Kannada	:	veelyade ele
Common Indian name	:	paan / pan

Distribution:

- A native of java, it is cultivated in india in assam, west Bengal, bihar,uttar pradesh, madya pradesh , maharastra, karnadaka, tamilnadu and kerala.

Botanical discribtion:

- Perennial, creeping herbs, stems semiwoods climbing by short adventitious root leaves bradly ovate, slightly cordate, acuminate (or) acute entire, globroas, pentiolata, mace spikes dense, cylindrical, female spikes 2.5-5 cm long pendulous fruits rarely produced often sunk in the fleshy spike, forming nodule like structure.

Parts used:

- Whole plant and leaf

Pharmacognosy:

- Leaves coriaccous, 10-18 by 5-12.5 cm broadly ovale acuminate, glabrous, 5-9 nerved the suprabasai nerves usually alternate base slightly cordate (or) usually rounded more (or) oblique, pentioles 1.3-2.5 cm.
- Microscopically leaves shows a dorsiventral structure. The cutice is hot striated, the hypodermis contains secretorycells, while the power epidermis bears cni to multicelluular hairs and ansiolytic stomata. The pentiole shows a discontinuous collencymas zone below the epidermis. Mucilage canals at the center, vascular bundles graded in size arranged in arcs and multicellular hairs.

Chemical constituents(phytochemical):

- Vitamin A &C, thiamine, ribatiavin nicotinic acid, glucose, fructose, maltose, sucrose malic acid, obalic acid, amino acids viz leucine phenylalanine, alanine, arginive, thrioniue, serineaspartic acid, glutamic acid, methioninive, valiue, thyrosine, asparagines, glucine, protine, ornithiue and α- amino butyric acid and β and α sitosterois, hentriacontane, pentatriacontane, n-triacontanol and stearic acid stigmasterol allylpyrocatechol, chevibetol acetate and allylpylrocatecol aiacetate, eugenol,carvacrol, chavicol, allylcatecol, chavibetol cineoue, estragol, eugenol hutuyl ether, P- Cunene, caryophylleve, candinene, α- terpinene and unidentified sequiterpenes, tocopuerol, eugenol, hydroxyl chavicol and mixture of two unideutified compounds β sitostercois, diosgenin (roots).

Pharmacological activities:

- Fungicidal
- Hematicidal
- Anti-baterial
- Anti-fungal
- Hypotensive
- Cardiac and respiratory depresent
- Anti- miotic
- Mild anti infertility
- Anthementic

- Anti tuberculous
- Anti-microbial
- Cardiotonic
- Stomach muscle retaxant

Action and uses:

- When leaf is chewed, the mild anti – infective content in the leaf. Fresuen breath and cleanse mouth. Its constituents directly enter the blood via buccal mulosa as this is a direct way of entry into blood stream, it is the best way to deliver drugs into the blood stream during sickness.
- The leaf contain several polsypuenois that not only figuts microbes, but also act as pain relivers and anti inflammatory agent. Recent studies have shown that the leaf contains tdnnis, sugar, diastage and an essential oil.
- A particular phenol called chavical p;resent in it has power ful antiseptic painful are for temporary relief. Betel leaves posses good diuretic properties. Therflre it can be mixed with dilute milk and consumed by sweetening as it helps ease urination.
- The betel leaf also beneficial in treating nervous pains an debility. The juice of the leaves, when consumed by adding a teaspoon of honey, twice a day act a good tonic.
- Betel leaves has been in use since ancient times for healing wounds. Ayurveda has hrougly belived in this property of the leaf. The juices of the leaves applied locally on the wound after which the betel leaf is wrapped around and bandaged. The wound is sure to heal with in a day or two.
- The paste of three paan leaves is taken with one gram rock salt with hot water. Thrice daily for filarial(shlee puta)the leaves juice is taken orally for indigestion and stomach disorders.
- The oral intake of leaves decreases cough and reducing swerling of throat and throat irritatation.
- Betel leaf juice is taken with honey for dry cough.It also helps in reducing difficulty in breathing for people suffering from asthma. Some apply mustard oil to the leave of the betel plant, warm it and keep it on the chest to bring relief from asthma

- Betel leaves are used for treating head ache and also to reduce the pain associated with arthritis. The juice from the leaves can be mixed with any hot oil (like coconut oil)and applied topically to treat lower back pain. The juice of the leaves is also used to relive headache.
- The leaves of the betel can increase alertness and mental functions in humans. Taking the juice of betel leaves with honey can help in curing nervous exhaustion.
- In Indonesia herbal tea made using betel leaves help in freeing the body from the unpleasant odor of sweating and menstrual. The herb also help in treating bad breath, intestinal worms, scanty urination, inflammation of testis, low breast milk production, diabetes, constipation palpitation, wounds ulcers and other skin inflammatory conditions.
- Dosage:For oral intake ayurvedic pharmacopeia of india recommended 10-20 ml juice of fresh leaves.
- In an animal study using betel leaves it was found that low dose of the herb can decrease T4 levels and increase T_3 levels.
- Also recent studies have shown that the leaf contains components that have chemo- preventive and anti-cancer properties.

3.3.3 LATERAL RESEARCH

Anti oxidant property:

The is reported das gupta and de(2004) evaluated the antioxidant activities of aqueous extract of the piper betel leaves by invitro methods.

- ❖ DPPH radicals scacenging activity
- ❖ Superoxide scavenging activity
- ❖ Hydroxyl radical scavenging activity

Finally the piper betelShowed very high antioxidant activity.

- ➢ Wong et al (2006) investicated the antioxidant properties of 25 edible trophical plants including pip;er betel using DPPH and FRAP assays. Total phenolic content was also estimated. Piper betel showed very high antioxidant activity.
- ➢ (rathee et al 2006) chevibetol, allylpyrocatechol and their respective glucosides were isolated from leaves. Among the isolated compounds allylpyrocatechol showed the best results in all the invitro exp;eriments. It could prevent Fe(II)induced lpo of liposoues and rat brain homogenates as well as gamma ray induced damage of PBR 322 plasmid DNA more efficient then chevibetol.
- ➢ Maniguha et al (2009) evaluated the antioxidant of piper betel leaves. Etuanolic extract of piper betel leaf showed strough antioxidant activities like reducing power DPPH radical, sup;eroxide anion scavenging and deoxyribose degradation actiovities when compared with different standard such as ascorbic acid, DMSO and BHI .

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ANTICANCER PROPERTY:

- ➢ The leaf constituents, hydroxyl chavicol and β carotene were found to reduce the number of papilomas per mouse
- ➢ Also the constituents, β carotene and α tocophenol are reported to significantly inhibit DMBA inducved skin tumour formation by 83-86 and 86% in swigs mice and 92.94 89% in male swigs bare mice respectively.
- ➢ While hydroxyl chavicol showed 90% ingibition in swigs bare mice.
- ➢ The constituent eugenol shows minimal protection in both the strains of mice.
- ➢ The leaf extract of betel quid when administered simultaneously while mutagenic tobacco specific N nitrosamines (present in the extract of chewed tobacco) viz N-

nitrosonornicotive (NNN) and 4 uretagenic affects and reduce the tumour incidence in mice.

- The leaf extract is reported to exhibit antitumor activity in 7, 12, dimethyl benzene and antnraceve (DMBA) treated wistar rats.

Anti microbial:

- The betel leaf oil posses strong antibacterial activity against the gram positive bacteria bacillus subtills , bacillus pumillus staphylococcus aurous, salmonella typhi, vibrio cholera and several other pathogenic microorganisms.
- The essential oil was also found to be more effective against tape worms(taenia solium) and hook worms(bunostomum trigonocephalum) than the synthetic anthelmentics, piperzive phosphate and hexyl resorcihol.

3.4. MILAGU *(மிளகு)*

3.4.1. GUNAPADAM ASPECT:

MILAGU SYNONYMS:

மிளகினுடப் பேர்தனையே விளம்பக்கேளு
முதிர்ந்து நின்ற திரை போக்கி மரிசியாகும்
வளகினுட வலசமுமா தீட்சணமாகும்
மகத்தானது வன்மாஞ் சியாமமாகுஞ்
குளகினுட மூஷ்ணமாம் சத்துவ நேஷங்
கோலக மாஞ்சரதுந் தனியுமாகும்
வளகினுட வாதத்தை யறுக்குகின்ற
மகத்தான மிளகுக்கு நாமமாமே

- Bogar Nigandu 1200

Thiraipokki

Marisi

Valasam

Thetsanam

Thuranam

Seyamam

Mooshnam

Sathuvanesam

Kolagam

Sarathunthini

Vadha Arukki

-Gunapadam Mooligai Vaguppu

சொல்லியதோர் அருட்டனென்றும் இதற்குப் பேரு
சொற்பெரிய மதங்கன் என்றும் பேருண்டாகும்
அல்லிய தோர் மலைத்திருக்க னென்றும் பேரு
அஷ்டமாசாதி யென்றும் இதற்குப் பேரு
கல்லியதோர் கத்திரிச னென்றும் பேரு
கருத்துரட னென்றும் நேர்வளந்தா னென்றும்
மல்லயதோர் கெந்தக னென்றிதற்குப் பேரு
வசனித்தோம் மிளகினிட அதீதப் பேரே.

- Panchakaviya Nigandu

- Arutan
- Mathengan
- Malaithirukkan
- Astamasathi
- Kathirisan
- Karvuthurudan
- Nerrvalandan
- Kanthakan

Properties:

Taste	:	Pitter, Pungent
Character	:	Veppam
Class	:	Pangent
used part	:	Seed
Action	:	

- Acrid
- Carminative
- Stimulant
- Antiperiodic
- Resolvent
- Rubefacient
- Antivatha
- Atidode.

Vernacular Names:

Tamil	:	Milagu
Eng	:	Pepper
Jehegu	:	Miriyalu
Malayam	:	Kurumilagu
Kannadam	:	Menasy
Sanskrit	:	Maricha
Hindi	:	Kali mirich
Persian	:	Filfliaisiah
Bengoli	:	Glomorich, Morich, Kalamorich
Gujarathi	:	Kalimori
Urdu	:	Fulfil Sioyah, Kalimirich

General Characters:

"சீதசுரம் பாண்டு சிலேஷ்மங் கிராணிமூலம்
வாதம் அருசிபித்தம் மாமூலம் ஓதுசன்னி
யாசம் அபஸ்மாரம் அடன்மேகம் காசம் இவை
நாசம் கறிமிளகி னால்"

"கோணுகின்ற பங்கவலி குய்யரோ கம்வாதம்
சோணிதங்க ழுத்திற்குள் தோன்றும் நோய் - காணரிய
காதுநோய் மாதர் குன்மங் காமாலை மந்தம் என்றீர்
ஏதுநோய் காய் இருக்கில் ஈங்கு.

It cures the Malarial Fever, Anemia, diarrhoea, piles, ulcer, Flattulance, Anorexia, Diabeties, cough, hemeplegia, vaginal disease, Neck and Nasal disorders, Jaundice, pitham, vatham, vedhasonitha Noi, and Sanni.

Therapeutic uses

- ❖ It is prescribed in cholareae, dyspepsia, flatulance, aliments.
- ❖ An infusion of black paper Forms a useful stimulant gargle in relaxed sore - throat and hoarseness dependent thereon and in tootheache also.
- ❖ Piperine is given with much benefit in aguee, gonorrhoea, haemorrhoids etc in doses of 3 to 10 grains.
- ❖ In Intermittent fever black pepper in doses of about a drachm is recommended to be given with the juice of leaves of ocimum sanctum or jeucas linifoha.
- ❖ The drug is also used in scorpion bite.

OTHER SIDDHA FORMULATIONS:

1. Milagu thiravagam
   Dosage : Kasu eadai
   Indication : Peptic ulcer, Indigestion, Anorexia
2. Milagu Legium
   Dosage : Punnai kai alavu
   Indication : Peptic ulcer, Vaivu, Diarrhoea.
3. Karuvepilai vadagam
   Dosage : Illanthai vithai alavu
   Indication : Anorexia, Diarrhoea, Discentry.

4. Kanthaga Chooranam

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, Indigestion, Constipation.

5. Sowbagiya sundi Chooranam

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, Indigestion, Diarrhoea.

6. Pirandai Vadagam

   Dosage : I Manthai vithai alavu

   Indication : Peptic ulcer, Vomitting, Anorexia

7. Thirikadgu Kirutham

   Dosage : 1 Spoon

   Indication : Piles, Diarrhoea.

8. Pirandai Chooranam

   Dosage : Verugadi alavu

   Indication : Peptic ulcer, Vomitting, piles, Abdominel, disorders.

9. Thirikadugu thiravagam

   Dosage : 5 Drops

   Indication : Peptic ulcer, Bronchial Asthma.

10. Muppirandai Chooranam

    Dosage : Verugadi alavu

    Indication : Peptic ulcer, Indigestion, Piles.

3.4.2. BOTANICAL ASPECTS

MILAGU (PIPER NIGRUM)

Taxonomical Classification:

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Superdivision	:	Spermatophyta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Subclass	:	Magnoliidae
Order	:	Piperales
Family	:	Piperaceae
Genus	:	*Piper* L.
Species	:	*Piper nigrum* L.

Common name:

Black pepper, white pepper, green pepper, peppercorn, Madagascar pepper (English); pippali (Sanskrit); kali mirch (Hindi, Urdu); milagu (Tamil)

Synonym:

Muldera multinervis Miq.

Habitat:

Montane tropical evergreen forest.

Geography and distribution

Black pepper is native to the Western Ghats of Kerala State in India, where it grows wild in the mountains.

It is cultivated all over the tropics as a commercial crop. Vietnam, Indonesia, Brazil and India are the major producers.

DESCRIPTION

Overview:

A climber that grows to a height or length of 10 m or more. Once the main stem is established it grows many side shoots to create a bushy column.

The plants form short roots, called adventitious roots, which connect to surrounding supports.

Leaves:

Almond-shaped, tapering towards the tip, dark green and shiny above, paler green below, arranged alternately on the stems.

Flowers:

Borne in clusters along flowering stalks known as spikes. 50–150 whitish to yellow-green flowers are produced on a spike.

Fruits:

Round, berry-like, up to 6 mm in diameter, green at first but turning red as they ripen, each containing a single seed. 50–60 fruits are borne on each spike.

Fruits are picked when green and immature to produce green pepper; when fully grown but still green and shiny to produce black pepper; and when slightly riper to produce white pepper (for which the fruits are also soaked to remove the fleshy outer layer).

Other pepper plants

Uses

Food

The fruits of *Piper nigrum* are used to make black pepper. This hotly pungent spice is one of the earliest known and most widely used spices in the world today. It is used as flavouring, particularly for savoury foods, meat dishes, sauces and snack foods. It is also used as a table condiment.

Black pepper, white pepper and green peppercorns are all produced from *Piper nigrum* fruits, but are harvested at different times and are processed differently.

India is a key producer of black pepper and exports much of what is grown. Peppercorns from Malabar and Tellicherry in Kerala, India, are particularly prized for their flavour and pungency.

Black pepper is also used to produce pepper oil and oleoresin, which are frequently used in the production of convenience foods and sometimes also for perfumery.

Of lesser importance is the use of preserved immature green pepper or fresh pepper fruits, which are eaten more like a vegetable.

Black peppercorns

Black peppercorns feature as remedies in Ayurveda, Siddha and Unani medicine in South Asia. They are most frequently used as an appetizer and to treat problems associated with the digestive system, particularly to eradicate parasitic worms. Some traditional uses of black pepper are supported by scientific evidence.

In Siddha medicine, black pepper has been used to aid digestion, improve appetite, treat coughs, colds, breathing and heart problems, colic, diabetes, anaemia and piles. Stomach ailments such as dyspepsia, flatulence, constipation and diarrhoea are all treated with black pepper, which may be mixed with other substances such as castor oil, cow's urine or ghee.

Black pepper has been prepared in tablet form as a remedy for cholera and syphilis, sometimes combined with other substances. It has also been used in tooth powder for toothache, and an infusion of black pepper has been suggested as a remedy for sore throat and hoarseness. Black pepper may be chewed to reduce throat inflammation.

Externally, it has been applied as a paste to boils and to treat hair loss and some skin diseases. Oil of pepper is reputed to alleviate itching. A mixture of sesame oil and powdered black pepper has been recommended for application to areas affected by paralysis. A mixture of black pepper and honey is regarded as a remedy for night blindness. Black pepper has been given by inhalation to comatose patients. It is also believed to be useful against hepatitis, urinary and reproductive disorders. In Ayurveda and Siddha medicine, a paste made using white pepper is applied to treat some eye diseases.

Phytochemical constitutents :

Black pepper contains about 3% essential oil, whose aroma is dominated (max. 80%) by monoterpenes hydrocarbons: sabinene, beta-pinene, limonene, furthermore terpinene, alpha-pinene, myrcene, delta3-carene and monoterpene derivatives (borneol, carvone, carvacrol, 1,8-cineol, linalool). Sesquiterpenes make up about 20% of the essential oil: beta-caryophyllene, humulene,beta-bisabolone and caryophyllene oxide and ketone. Phenylether (eugenol, myristicin, safrole) are found in traces. Loss of monoterpenes due to bad storage conditions (especially for ground pepper) should be avoided.

Pharmacological activity:

- **Constituents**: Black pepper has been found to contain piperine[1], alkamides[9], piptigrine[7], wisanine[7], dipiperamide D[10], and dipiperamide E[10].
- **Acetylcholinesterase inhibitory activity**: In an *in vitro* study, an extract of *Piper nigrum* L. seeds showed 50-65% inhibitory activity on acetylcholinesterase.[2]
- **Antibacterial effects**: In an *in vitro* study using 12 different genera of bacterial populations isolated from the oral cavity of 200 individuals, an aqueous decoction of black pepper (*Piper nigrum* L.) exhibited 75% antibacterial activity as compared to aqueous decoction of bay leaf (53.4%) and aqueous decoction of aniseed (18.1%), at the concentration of 10mL/disc.[4]
- **Anti-inflammatory effects**: Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica*, *Albizia lebbeck*, *Piper nigrum*, *Zingiber officinale*, and *Piper longum* demonstrated 31.3% inhibition against carrageenan-induced acute inflammation in Wistar Albino rats, while ibuprofen (50 mg/kg orally) exerted 68.1% inhibition.[3] Aller-7 also exhibited a dose-dependent (150-350mg/kg) anti-inflammatory effect against Freund's adjuvant-induced arthritis in Wistar Albino rats; an approximately 63% inhibitory effect was observed at a dose of 350mg/kg.
- **Antilarval activity**: Piptigrine, isolated from the dried ground seeds of *Piper nigrum* Linn., exhibited toxicity of 15.0ppm against fourth instar larvae of *Aedes aegypti* Liston.[7]
- **Antioxidant effects**: Based on animal study, a polyherbal formulation (Aller-7/NR-A2) containing extracts from seven medicinal plants including *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellerica*, *Albizia lebbeck*, *Piper nigrum*, *Zingiber officinale*, and *Piper longum*exhibited concentration-dependent scavenging activities toward biochemically generated hydroxyl radicals (IC_{50} 741.73mcg/mL); superoxide anion (IC_{50} 24.65mcg/mL by phenazine methosulfate-nicotinamide adenine dinucleotide [PMS-NADH] assay and IC_{50}4.27mcg/mL by riboflavin/nitroblue tetrazolium [NBT] light assay), nitric oxide (IC_{50} 16.34mcg/mL); 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical (IC_{50} 5.62mcg/mL); and 2,2-azinobis-ethyl-benzothiozoline-sulphonic acid diammonium salt (ABTS) radical (IC_{50} 7.35mcg/mL).[5]Aller-7 inhibited free radical-

induced hemolysis in the concentration range of 20-80mcg/mL. Aller-7 also significantly inhibited nitric oxide release from lipopolysaccharide-stimulated murine macrophages.

- **Cytochrome P (CYP) 450 effects**: In *in vitro* studies, constituents isolated from *Piper nigrum*, including piperine and dipiperamides D and E, potently inhibited some CYP450 metabolic pathways, including CYP2D6[9] and CYP3A4[8,10].
- **Gastrointestinal effects**: In a clinical study of intestinal peristalsis in 16 healthy volunteers, consumption of 1.5g of black pepper in capsules increased the orocecal transit time from 90 ± 51 minutes to 122 ± 88 minutes (p=0.09).[11] In an *in vitro* study, piperine inhibited digoxin and cyclosporine A transport in Caco-2 cells with IC_{50} values of 15.5 and 74.1mcM, respectively.[8] The bactericidal and anti-adhesive properties of black pepper have also been investigated against *Helicobacter pylori*, however, aqueous extracts did not show bactericidal effect on any of the isolates.[6]
- **Neural effects**: In an *in vitro* study using whole-cell patch-clamp electrophysiology, piperine, a pungent alkaloid found in black pepper, had similar agonist effects on the human vanilloid receptor TRPV1 as capsaicin.[1] However, piperine could induce greater receptor desensitization and exhibit a greater efficacy than capsaicin.

3.4.3. LATERAL RESEARCH :

Black pepper (Piper nigrum L.) native of south India popularly known as" king of spices". Pepper is mostly used in the curry recipes as masalas and also as ingredient in the prescriptions of folk medicine, Ayurveda and traditional medicinal systems. The spicy tang of pepper is due to the presence of piperamides which are the pungent bioactive alkaloids accumulate in the skin and seeds of the fruit. Among them piperine is the major chemical constituent responsible for the bitter taste of the black pepper.

In the present study piperine was evaluated for its antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Alternaria alternata, Aspergillus niger, Aspergillus flavus and Fusarium oxysporum. The antibacterial activity was measured by agar well diffusion method and antifungal activity by poisoned food technique. Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm. maximum zone of inhibition was against Gram positive bacteria Staphylococcus aureus (18mm) and minimum against Gram negative bacteria Escherichia coli (8mm). Piperine showed maximum antifungal activity towards Fusarium oxysporum (14mm) and very least effect against Aspergillus niger (38mm). The results showed significant activity of piperine and suggesting its use as natural antimicrobial agent.

3.5. BREAST MILK

3.5.1. GUNAPADAM ASPECT

தன்னியாமென் றோதிச் சருவதோ ஷங்களும் போம்
உன்னிய தாபமொழியுங்காண் - சன்னியோடு
வாதசுரம் பித்தசுரம் வன்கபச் சுரந்தணியுங்
கோதில் வலுமையுண்டாங் கூறு

இருதோசம்போக்கும் மிகற் கிரிச்சந் தீர்க்கும்
அருந்து மருந்தி னனுபானம் - பொருந்தும்
அஞ்சனத்திற் கழகு மணல் வறட்சி நீங்கிவிடும்
பஞ்சினடி மாதர் முலைப்பால்.

Breast milk is an ideal diet for children as it contains all the nutrients essential for the growth of children and is easily digestible. Children feed on breast milk will rarely contract infectious diseases.

Mother's, while giving breast milk to their babies, and also bestows best qualities such as kindness, affection, bondness and knowledge.

Mother's milk is prescribed as best adjuvant for certain diseases. For chronic constipation caused in delirium and fever cases, breast milk is considered as best medicine.

Milk is ground with borne camphor, saffron flower, Cloves and stem of betel leaf. A cloth soaked in this milk is applied over the body and head for the control of fever and headache.

Breast milk soaked in a cloth and applied over the abdomen, cures dysuria and constipation especially in children.

Breast milk is also instilled as eye drops for eye diseases.

Breast milk is used to purify cinnabar, rasa chendooram, sub-chloride of mercury etc. It is also useful for grinding the drugs like chandamaarutha chendooram and kasturi pills. It is also added in the preparation of saranaathi thailam.

Breast milk and ginger juice are taken one part and equal quantity of gingerly oil is mixed, boiled and used as a hair oil for curing the headache caused by cold.

3.5.2. SCIENTIFIC ASPECT

The team of researcher isolated the key compound - a protein called alpha-lactalbumin.Subsequent tests showed the compound becomes lethal only when exposed to acid, as it is in the stomach, so the scientists mixed it with oleic acid, which is found in babies' stomachs, to form a compound they call HAMLET (human alphalactalbumin made lethal to tumour cells).

The Swedish team, led by Professor Catharina Svanborg, have shown that HAMLET attacks cancer cells, causing apoptosis - a form of cell suicide - in 40 kinds of tumour.

Studies with rats showed that after just seven weeks a highly invasive brain cancer called glioblastoma was seven times smaller in those treated with HAMLET.
The product has also been made into a cream and tested on warts (which share the same growth properties as tumours) and found to reduce their size by 75 per cent in 20 volunteers.

DIABETES & PARKINSON'S

Breast milk could be a new, and easier, source of stem cells. Stem cells are one of the most exciting discoveries in medicine, thanks to their remarkable ability to develop into many different cell types in the body, serving as a sort of internal repair system.

Stem cells are already being used to treat leukaemia and could soon help treat eye conditions.

Scientists are also researching their potential in the longer term for treating conditions such as spinal injuries, diabetes and Parkinson's disease.

A molecular biologist at Perth University, Australia, has discovered stem cells in breast milk.

Dr Mark Cregan and his team cultured the cells of human breast milk and found the result was positive for a stem cell marker called nestin.

'These cells have all the physical characteristics of stem cells,' he says. 'What we will do next is to see if they behave like stem cells.'

If so, this promises to provide researchers with an ethical and easier means of harvesting stem cells for researching treatments. Indeed, Dr Cregan believes this development could be possible within five years.

DIARRHOEA

Chronic diarrhoea kills up to 2.2 million people worldwide every year, mostly children in developing countries. Scientists are looking at whether breast milk could help treat it.

One approach is based on indigestible sugars known as oligosaccharides, many of which occur only in human milk. These sugars protect a baby from pathogens to which the mother has never been exposed.

It's thought oligosaccharides might be used to boost elderly people's weakened natural protection against pathogens. They could also be used after a course of strong antibiotics by helping re-colonise the digestive tract with beneficial bacteria. So far, scientists have been able to genetically engineer mice to produce oligosaccharides in their milk and are working on bioengineering bacteria to produce human oligosaccharides to put into baby formula milk (to protect bottle-fed babies) or as supplements for adults.

Other compounds found in breast milk, called lysozyme and lactoferrin, have been tested on children with diarrhoea and have been shown to not only be an effective treatment, but to offer some sort of protection against future bouts.

ARTHRITIS

Breast milk contains lactoferrin, which helps prevent babies' immune systems from overreacting.

This is being looked as a potential treatment for auto-immune conditions, such as rheumatoid arthritis, multiple sclerosis and septic shock.

In Italy, studies are under way to see if a breast milk molecule called glyerophosphocholine (GPC) can improve mental function in people with dementia or victims of stroke and traumatic brain injury.

In many separate trials, GPC appears to improve memory, attention and orientation in people with various forms of dementia, including Alzheimer's.

It works like a brain nutrient, feeding the most energetically needy cells of our body, such as the brain cells.

ACNE

A science student at the University of California recently discovered that the lauric acid in breast milk reduces irritation and spots, and has developed an acne cream that is undergoing clinical trials.

The cream uses tiny gold particles to carry lauric acid into pores where its anti-microbial properties fight bacteria.

As breast milk is difficult to source, researchers are working to develop new sources for its healthgiving compounds.

The compounds lysozyme and lactoferrin are harvested for research from a specific variety of rice, and the milk from genetically engineered goats and cows.

Though some of these beneficial compounds are found in milk from other animals, others occur only in human milk, and the nonhuman versions are less potent when given to humans.

3.5.3. LATERAL RESEARCH

The benefits of breast milk are well known, but why breastfeeding protects against various forms of cancer remains a mystery. This study found high levels of cancer-fighting TNF-related apoptosis inducing ligand (TRAIL) in human milk, which might be one source of breast milk's anticancer activity. Researchers took samples of colostrum, the first milk available to newborns, and of mature breast milk from new mothers. Researchers then obtained samples of blood from healthy women, and various ready-to-feed infant formulas. The colostrum, mature breast milk, blood and formula were then all tested to measure their level of TRAIL. The researchers found that colostrum and breast milk contained 400- and 100-fold, respectively, higher levels of TRAIL than blood. No TRAIL was detected in the formula.

"The important role of breastfeeding in the prevention of certain childhood cancers, such as lymphoblastic leukemia, Hodgkin's disease, and neuroblastoma, has been previously demonstrated," wrote the authors. "However, endogenous soluble TRAIL represents a strong candidate to explain the overall biological effect of breastfeeding against cancer."

Background: TNF-related apoptosis inducing ligand (TRAIL) is a pleiotropic cytokine, which plays a key role in the immune system as well as in controlling the balance of apoptosis and proliferation in various organs and tissues.

Objective: To investigate the presence and levels of soluble TRAIL in human colostrum and milk.

Methods: The levels of soluble human TRAIL were measured in human colostrum (day 2 after delivery) and breast milk (day 5 after delivery). The presence of TRAIL was also measured in infant formula.

Results: Levels of soluble TRAIL in the colostrum and mature human milk were, respectively, at least 400 and 100 fold higher than those detected in human serum. No TRAIL was detected in formula.

Conclusion: Human soluble TRAIL is present at extremely high levels in human colostrum and human milk and might have a significant role in mediating the anti-cancer activity of human milk.

3.6.DISEASE REVIEW

3.6.1. SIDDHA ASPECT:

புற்று வரும் வழி

உரைபத னழிந்த உணவுகளு ண்ணல்
வரைபடு புலால் மீன் வழக்கத் துண்ணல்
புணர்ச்சி மிகுதலின் வெப்ப மீறுதல்
வெப்ப நிலைகள் வேறுபடுபவரை
அடிக்கடி புணர்தல் ஒழுக்கு நோயுறல்
உடலழக்கேலுதல் அணுக்கள் புகுதல்
எனுமிவை பிறவும் பலவகைப்பட்ட
புற்றுநோய்க் கடிப்படையாகும்.
- மான்முருகியம் என்றும் தமிழ் மருத்துவநூல்

புற்றின் முற்குறி

புற்றுத் தோன்றிடந் தினவு தோன்றல்
அவ்விடம் புரைபட்டெனத் தோன்றிடுதல்
தசைகழிந் தகவல் தளர்ச்சி தோன்றல்
உடல்நிலை வேறுபட் டென்னத் தோன்றல்
புற்றும் உறுப்பின் தொழில் குறைந்திடுதல்
ஆற்றல் கெடுதல் உறுப்பு நோதல்
சிவத்தல் கடுத்தல் குத்தல் பிறவும்
புற்றுநோயின் முன்னமாகும்

புற்றின் குறிகுணம்

சிறுக சிறுகச் சேவற் பூப்போல
கூர்ந்து திரண்டு மழி தசை வளர்தல்
பிணியுற் றிடமும் அயலும் நரம்பும்
மிகவும் நோதல் தசையழிந் திடுதல்
நரம்பு முதலிய அரிக்கப்படுதல்
குருதியும் பழுப்பு தோன்றல் ஒழுகல்
உடம்பு மெலிதல் தடுமாற் றயர்ச்சி
எனுமிவை பிறவும் புற்றின் குறியே

புரைதோன்றுதலும் தசை திறைத் திடலும்
உட்சதை திரளலும் குருதியொழுகலும்
எளிதிற் றீராப் புண் தோன்றிடலும்
குருக்கள் போலத் தசைமேற் றொன்றலும்

உணவு றாமை தொடர்ந்த தோன்றலும்
அடிக்கடி யிருமல் குரலடை தோன்றலும்
புள்ளிகள் தோன்றலும் நிறம் வேறுபடுதலும்
நீங்காத் தளர்ச்சி யயர்ச்சி தோன்றலும்
குடற்பிணி தொடர்ந்த தோன்றலும் பிறவும்
புற்றுநோய்க்கும் முன்னமாதலின்
முற்பட வுணர்ந்து போதல் மாமே

அல்குல்புற்றின் குணம்

அல்குல் வாயினு மகந்து மருகினும்
சேவற்பூப்போல் அழிசதை வளர்தல்
நோதல் குத்தல் சீழ்மிக வொழுகல்
குருதி வெளிப்படல் தசைநரம் பழிதல்
புரைபடல் எனுமிவை பெண்குறிப் புற்றின்
குறியென மொழிப நெறிபறி புலவர்

தீரும், தீரா நோய்க்குறி

புற்று நோயனைத்தும் பொருந்தப் பேணின்
அரிதிற் றீர்வன வாகுமென்ப
அறுவையிற் சிற்சில அகல்வது மாகும்
மிக்க தளர்ச்சி மெலிவு நீர் வேட்கை
பொறிகளின் ஆற்றலழிதல் மயக்கம்
காய்ச்சல் தோன்றல் விக்கலெடுத்தல்
உணவு செல்லாமை உண்டத றாமை
எனுமிவை பிறவுந் தீராக் குறியும்

3.6.2. MODERN ASPECT:

INTRODUCTION

Cervical cancer is a cancer arising from the cervix. It is due to the abnormal growth of cells that have the ability to invade or spread to other parts of the body. Early on, typically no symptoms are seen. Later symptoms may include abnormal vaginal bleeding, pelvic pain, or pain during sexual intercourse. While bleeding after sex may not be serious, it may also indicate the presence of cervical cancer.

Human papillomavirus (HPV) infection appears to be involved in the development of more than 90% of cases; most people who have had HPV infections, however, do not develop cervical cancer. Other risk factors include smoking, a weak immune system,birth control pills, starting sex at a young age, and having many sexual partners, but these are less important. Cervical cancer typically develops from precancerous changes over 10 to 20 years. About 90% of cervical cancer cases are squamous cell carcinomas, 10% are adenocarcinoma, and a small number are other types. Diagnosis is typically by cervical screening followed by a biopsy. Medical imaging is then done to determine whether or not the cancer has spread.

HPV vaccines protect against between two and seven high-risk strains of this family of viruses and may prevent up to 90% of cervical cancers. As a risk of cancer still exists, guidelines recommend continuing regular Pap smears. Other methods of prevention include: having few or no sexual partners and the use of condoms. Cervical cancer screening using the Pap smear oracetic acid can identify precancerous changes which when treated can prevent the development of cancer. Treatment of cervical cancer may consist of some combination of surgery, chemotherapy, and radiotherapy. Five year survival rates in the United States are 68%. Outcomes, hever, depend very much on how early the cancer is detected.[8]

Worldwide, cervical cancer is both the fourth-most common cause of cancer and the fourth-most common cause of death from cancer in women. In 2012, an estimated 528,000 cases of cervical cancer occurred, with 266,000 deaths. This is about 8% of the total cases and total deaths from cancer. About 70% of cervical cancers occur in developing countries. In low-income countries, it is the most

common cause of cancer death. In developed countries, the widespread use of cervical screening programs has dramatically reduced rates of cervical cancer. In medical research, the most famous cell line known as HeLa was developed from cervical cancer cells of a woman named Henrietta Lacks.

Signs and symptoms

The early stages of cervical cancer may be completely free of symptoms. Vaginal bleeding, contact bleeding (one most common form being bleeding after sexual intercourse), or (rarely) a vaginal mass may indicate the presence of malignancy. Also, moderate pain during sexual intercourse and vaginal discharge are symptoms of cervical cancer. In advanced disease, metastases may be present in the abdomen, lungs, or elsewhere.

Symptoms of advanced cervical cancer may include: loss of appetite, weight loss, fatigue, pelvic pain, back pain, leg pain, swollen legs, heavy vaginal bleeding, bone fractures, and/or (rarely) leakage of urine or feces from the vagina. Bleeding after douching or after a pelvic exam is a common symptom of cervical cancer.

Causes

Infection with some types of HPV is the greatest risk factor for cervical cancer, followed by smoking. HIV infection is also a risk factor. Not all of the causes of cervical cancer are known, however, and several other contributing factors have been implicated.

Human papillomavirus

Human papillomavirus types 16 and 18 are the cause of 75% of cervical cancer cases globally, while 31 and 45 are the causes of another 10%.

Women who have many sexual partners (or who have sex with men who have had many other partners) have a greater risk.

Of the 150-200 types of HPV known, 15 are classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), three as probable high-risk (26, 53, and 66), and 12 as low-risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108).

Genital warts, which are a form of benign tumor of epithelial cells, are also caused by various strains of HPV. However, these serotypes are usually not related to cervical cancer. It is common to have multiple strains at the same time, including those that can cause cervical cancer along with those that cause warts.

Infection with HPV is generally believed to be required for cervical cancer to occur.

Smoking

Cigarette smoking, both active and passive, increases the risk of cervical cancer. Among HPV-infected women, current and former smokers have roughly two to three times the incidence of invasive cancer. Passive smoking is also associated with increased risk, but to a lesser extent. Smoking has also been linked to the development of cervical cancer. Smoking can increase the risk in women a few different ways, which can be by direct and indirect methods of inducing cervical cancer. A direct way of contracting this cancer is a smoker has a higher chance of CIN3 occurring which has the potential of forming cervical cancer. When CIN3 lesions lead to cancer, most of them have the assistance of the HPV virus, but that is not always the case, which is why it can be considered a direct link to cervical cancer.Heavy smoking and long-term smoking seem to have more of a risk of getting the CIN3 lesions than lighter smoking or not smoking at all. Although smoking has been linked to cervical cancer, it aids in the development of HPV which is the leading cause of this type of cancer. Also, not only does it aid in the development of HPV, but also if the woman is already HPV-positive, she is at an even greater likelihood of contracting cervical cancer.

Oral contraceptives

Long-term use of oral contraceptives is associated with increased risk of cervical cancer. Women who have used oral contraceptives for 5 to 9 years have about three times the incidence of invasive cancer, and those who used them for 10 years or longer have about four times the risk.

Multiple pregnancies

Having many pregnancies is associated with an increased risk of cervical cancer. Among HPV-infected women, those who have had seven or more full-term pregnancies have around four times the risk of cancer compared with women with no pregnancies, and two to three times the risk of women who have had one or two full-term pregnancies.

Diagnosis

Biopsy

The Pap smear can be used as a screening test, but is false negative in up to 50% of cases of cervical cancer. Confirmation of the diagnosis of cervical cancer or precancer requires a biopsy of the cervix. This is often done through colposcopy, a magnified visual inspection of the cervix aided by using a dilute acetic acid (e.g. vinegar) solution to highlight abnormal cells on the surface of the cervix. Medical devices used for biopsy of the cervix include punch forceps, SpiraBrush CX, SoftBiopsy, or Soft-ECC.

Colposcopic impression, the estimate of disease severity based on the visual inspection, forms part of the diagnosis.

Further diagnostic and treatment procedures are loop electrical excision procedure and conization, in which the inner lining of the cervix is removed to be examined pathologically. These are carried out if the biopsy confirms severe cervical intraepithelial neoplasia.

This large squamous carcinoma (bottom of picture) has obliterated the cervix and invaded the lower uterine segment. The uterus also has a roundleiomyoma up higher.

Often before the biopsy, the doctor asks for medical imaging to rule out other causes of woman's symptoms. Imaging modalities such as ultrasound, CT scan and MRI have been used to look for alternating disease, spread of tumor and effect on adjacent structures. Typically, they appear as heterogeneous mass in the cervix.

Precancerous lesions

Cervical intraepithelial neoplasia, the potential precursor to cervical cancer, is often diagnosed on examination of cervical biopsies by a pathologist. For premalignant dysplastic changes, cervical intraepithelial neoplasia grading is used.

The naming and histologic classification of cervical carcinoma precursor lesions has changed many times over the 20th century. TheWorld Health Organization classification system was descriptive of the lesions, naming them mild, moderate, or severe dysplasia orcarcinoma in situ (CIS). The term, cervical intraepithelial neoplasia (CIN) was developed to place emphasis on the spectrum of abnormality in these lesions, and to help standardise treatment. It classifies mild dysplasia as CIN1, moderate dysplasia as CIN2, and severe dysplasia and CIS as

CIN3. More recently, CIN2 and CIN3 have been combined into CIN2/3. These results are what a pathologist might report from a biopsy.

These should not be confused with the Bethesda system terms for Pap smear (cytopathology) results. Among the Bethesda results: Low-grade Squamous Intraepithelial Lesion (LSIL) and High-grade Squamous Intraepithelial Lesion (HSIL). An LSIL Pap may correspond to CIN1, and HSIL may correspond to CIN2 and CIN3, however they are results of different tests, and the Pap smear results need not match the histologic findings.

Cancer subtypes

Histologic subtypes of invasive cervical carcinoma include the following: Though squamous cell carcinoma is the cervical cancer with the most incidence, the incidence of adenocarcinoma of the cervix has been increasing in recent decades.

- squamous cell carcinoma (about 80-85%)
- adenocarcinoma (about 15% of cervical cancers)
- adenosquamous carcinoma
- small cell carcinoma
- neuroendocrine tumour
- glassy cell carcinoma
- villoglandular adenocarcinoma

Noncarcinoma malignancies which can rarely occur in the cervix include melanoma and lymphoma. The FIGO stage does not incorporatelymph node involvement in contrast to the TNM staging for most other cancers.

For cases treated surgically, information obtained from the pathologist can be used in assigning a separate pathologic stage, but is not to replace the original clinical stage.

Staging

Cervical cancer is staged by the International Federation of Gynecology and Obstetrics (FIGO) staging system, which is based on clinical examination, rather than surgical findings. It allows only these diagnostic tests to be used in determining the stage: palpation, inspection,colposcopy, endocervical curettage, hysteroscopy, cystoscopy, proctoscopy, intravenous urography, and X-ray examination of the lungs and skeleton, and cervical conization.

Prevention

Screening

Checking the cervix by the Papanicolaou test, or Pap smear, for cervical cancer has been credited with dramatically reducing the number of cases of and mortality from cervical cancer in developed countries. Pap smear screening every 3–5 years with appropriate follow-up can reduce cervical cancer incidence up to 80%. Abnormal results may suggest the presence of precancerous changes, allowing examination and possible preventive treatment. The treatment of low-grade lesions may adversely affect subsequent fertility and pregnancy. Personal invitations encouraging women to get screened are effective at increasing the likelihood they will do so. Educational materials also help increase the likelihood women will go for screening, but they are not as effective as invitations.

According to the 2010 European guidelines, the age at which to start screening ranges between 20 and 30 years of age, "but preferentially not before age 25 or 30 years", and depends on burden of the disease in the population and the available resources.

In the United States, screening is recommended to begin at age 21, regardless of age at which a woman began having sex or other risk factors. Pap tests should be done every three years between the ages of 21 and 65. In women over the age of 65, screening may be discontinued if no abnormal screening results were seen within the previous 10 years and no history of CIN 2 or higher exists. HPV vaccination status does not change screening rates. Screening can occur every 5 years between ages 30 and 65 when a combination of cervical cytology screening and HPV testing is used and this is preferred. However, it is acceptable to screen this age group with a Pap smear alone every 3 years. Screening is not beneficial before age 25 as the rate of disease is low. Screening is not beneficial in women older than 60 years if they have a history of negative results.

Liquid-based cytology is another potential screening method. Although it was probably intended to improve on the accuracy of the Pap test, its main advantage has been to reduce the number of inadequate smears from around 9% to around 1%. This reduces the need to recall women for a further smear. The United States Preventive Services Task Force supports screening every 5 years in those who are between 30 and 65 years when cytology is used in combination with HPV testing.

Pap smears have not been as effective in developing countries. This is in part because many of these countries have an impoverished health care infrastructure, too few trained and skilled professionals to obtain and interepret Pap smears, uninformed women who get lost to follow-up, and a lengthy turn-around time to get results. These realities have resulted in the investigation of cervical screening approaches that use fewer resources and offer rapid results such as visual inspection with acetic acid or HPV DNA testing.

Barrier protection

Barrier protection and/or spermicidal gel use during sexual intercourse decreases cancer risk. Condoms offer protection against cervical cancer. Evidence on whether condoms protect against HPV infection is mixed, but they may protect against genital warts and the precursors to cervical cancer. They also provide protection against other STIs, such as HIV and Chlamydia, which are associated with greater risks of developing cervical cancer.

Condoms may also be useful in treating potentially precancerous changes in the cervix. Exposure to semen appears to increase the risk of precancerous changes (CIN 3), and use of condoms helps to cause these changes to regress and helps clear HPV. One study suggests that prostaglandin in semen may fuel the growth of cervical and uterine tumors and that affected women may benefit from the use of condoms.
Abstinence also prevents HPV infection.

Vaccination

Two HPV vaccines (Gardasil and Cervarix) reduce the risk of cancerous or precancerous changes of the cervix and perineum by about 93% and 62%, respectively. The vaccines are between 92% and 100% effective against HPV 16 and 18 up to at least 8 years.

HPV vaccines are typically given to age 9 to 26 as the vaccine is only effective if given before infection occurs. The vaccines have been shown to be effective for at least 4 to 6 years, and they are believed to be effective for longer; however, the duration of effectiveness and whether a booster will be needed is unknown. The high cost of this vaccine has been a cause for concern. Several countries have considered (or are considering) programs to fund HPV vaccination.
Since 2010, young women in Japan have been eligible to receive the cervical cancer vaccination for free. In June 2013, the Japanese Ministry of Health, Labor and Welfaremandated that, before administering the vaccine, medical institutions must

inform women that the Ministry does not recommend it. However, the vaccine is still available at no cost to Japanese women who choose to accept the vaccination.

Nutrition

Vitamin A is associated with a lower risk[61] as are vitamin B12, vitamin C, vitamin E, and beta-carotene.[62]

Treatment

The treatment of cervical cancer varies worldwide, largely due to access to surgeons skilled in radical pelvic surgery, and the emergence of "fertility-sparing therapy" in developed nations. Because cervical cancers are radiosensitive, radiation may be used in all stages where surgical options do not exist.

Microinvasive cancer (stage IA) may be treated by hysterectomy (removal of the whole uterus including part of the vagina For stage IA2, the lymph nodes are removed, as well. Alternatives include local surgical procedures such as a loop electrical excision procedure or cone biopsy. For 1A1 disease, a cone biopsy (cervical conization) is considered curative.

If a cone biopsy does not produce clear margins (findings on biopsy showing that the tumor is surrounded by cancer free tissue, suggesting all of the tumor is removed), one more possible treatment option for women who want to preserve their fertility is a trachelectomy. This attempts to surgically remove the cancer while preserving the ovaries and uterus, providing for a more conservative operation than a hysterectomy. It is a viable option for those in stage I cervical cancer which has not spread; however, it is not yet considered a standard of care, as few doctors are skilled in this procedure. Even the most experienced surgeon cannot promise that a trachelectomy can be performed until after surgical microscopic examination, as the extent of the spread of cancer is unknown. If the surgeon is not able to microscopically confirm clear margins of cervical tissue once the woman is under general anesthesia in the operating room, a hysterectomy may still be needed. This can only be done during the same operation if the woman has given prior consent. Due to the possible risk of cancer spread to the lymph nodes in stage 1b cancers and some stage 1a cancers, the surgeon may also need to remove some lymph nodes from around the uterus for pathologic evaluation.

A radical trachelectomy can be performed abdominally or vaginally and opinions are conflicting as to which is better. A radical abdominal trachelectomy with lymphadenectomy usually only requires a two- to three-day hospital stay, and most

women recover very quickly (about six weeks). Complications are uncommon, although women who are able to conceive after surgery are susceptible to preterm labor and possible late miscarriage. Wait at least one year is generally recommended before attempting to become pregnant after surgery. Recurrence in the residual cervix is very rare if the cancer has been cleared with the trachelectomy. Yet, women are recommended to practice vigilant prevention and follow-up care including Pap screenings/colposcopy, with biopsies of the remaining lower uterine segment as needed (every 3–4 months for at least 5 years) to monitor for any recurrence in addition to minimizing any new exposures to HPV through safe sex practices until one is actively trying to conceive.

Early stages (IB1 and IIA less than 4 cm) can be treated with radical hysterectomy with removal of the lymph nodes or radiation therapy. Radiation therapy is given as external beam radiotherapy to the pelvis and brachytherapy (internal radiation). Women treated with surgery who have high-risk features found on pathologic examination are given radiation therapy with or without chemotherapy to reduce the risk of relapse.

Brachytherapy for cervical cancer

Larger early-stage tumors (IB2 and IIA more than 4 cm) may be treated with radiation therapy and cisplatin-based chemotherapy, hysterectomy (which then usually requires adjuvant radiation therapy), or cisplatin chemotherapy followed by hysterectomy. When cisplatin is present, it is thought to be the most active single agent in periodic diseases.

Advanced-stage tumors (IIB-IVA) are treated with radiation therapy and cisplatin-based chemotherapy. On June 15, 2006, the US Food and Drug Administration approved the use of a combination of two chemotherapy drugs, hycamtin and cisplatin, for women with late-stage (IVB) cervical cancer treatment. Combination treatment has significant risk of neutropenia, anemia, and thrombocytopenia side effects.

For surgery to be curative, the entire cancer must be removed with no cancer found at the margins of the removed tissue on examination under a microscope. This procedure is known as exenteration

Prognosis

Prognosis depends on the stage of the cancer. The chance of a survival rate around 100% is high for women with microscopic forms of cervical cancer. With

treatment, the five-year relative survival rate for the earliest stage of invasive cervical cancer is 92%, and the overall (all stages combined) five-year survival rate is about 72%. These statistics may be improved when applied to women newly diagnosed, bearing in mind that these outcomes may be partly based on the state of treatment five years ago when the women studied were first diagnosed. With treatment, 80 to 90% of women with stage I cancer and 60 to 75% of those with stage II cancer are alive 5 years after diagnosis. Survival rates decrease to 30 to 40% for women with stage III cancer and 15% or fewer of those with stage IV cancer 5 years after diagnosis.

According to the International Federation of Gynecology and Obstetrics, survival improves when radiotherapy is combined with cisplatin-based chemotherapy.

As the cancer metastasizes to other parts of the body, prognosis drops dramatically because treatment of local lesions is generally more effective than whole-body treatments such as chemotherapy.

Interval evaluation of the woman after therapy is imperative. Recurrent cervical cancer detected at its earliest stages might be successfully treated with surgery, radiation, chemotherapy, or a combination of the three. About 35% of women with invasive cervical cancer have persistent or recurrent disease after treatment.

Average years of potential life lost from cervical cancer are 25.3. Around 4,600 women were projected to die in 2001 in the US of cervical cancer, and the annual incidence was 13,000 in 2002 in the US, as calculated by SEER. Thus, the ratio of deaths to incidence is about 35.4%.

Regular screening has meant that precancerous changes and early-stage cervical cancers have been detected and treated early. Figures suggest that cervical screening is saving 5,000 lives each year in the UK by preventing cervical cancer. About 1,000 women per year die of cervical cancer in the UK. All of the Nordic countries have cervical cancer-screening programs in place. Pap smear was integrated into clinical practice in the Nordic countries in the 1960s.

Epidemiology

Worldwide

Worldwide, cervical cancer is both the fourth-most common cause of cancer and deaths from cancer in women. In 2012, 528,000 cases of cervical cancer were estimated to have occurred, with 266,000 deaths. It is the second-most common cause of female-specific cancer after breast cancer, accounting for around 8% of both total

cancer cases and total cancer deaths in women. About 80% of cervical cancers occur in developing countries.

United States

An estimated 12,900 new cervical cancers and 4,100 cervical cancer deaths will occur in the United States in 2015. In the United States, it is the eight-most common cancer of women. The median age at diagnosis is 48. Hispanic women are significantly more likely to be diagnosed with cervical cancer than the general population. In 1998, about 12,800 women were diagnosed in the US and about 4,800 died. In 2014, an estimated 12,360 new cases were expected to be diagnosed, and about 4,020 were expected to die of cervical cancer. Among cancers of the female reproductive tract it is less common than endometrial cancer and ovarian cancer. The rates of new cases in the United States was 7 per 100,000 women in 2004. Cervical cancer deaths decreased by approximately 74% in the last 50 years, largely due to widespread Pap smear screening. The annual direct medical cost of cervical cancer prevention and treatment prior to introduction of the HPV vaccine was estimated at $6 billion.

EU

In the European Union, about 34,000 new cases per year and over 16,000 deaths due to cervical cancer occurred in 2004.

UK

Cervical cancer is the 12th-most common cancer in women in the UK (around 3,100 women were diagnosed with the disease in 2011), and accounts for 1% of cancer deaths (around 920 died in 2012). With a 42% reduction from 1988-1997, the NHS-implemented screening programme has been highly successful, screening the highest-risk age group (25–49 years) every 3 years, and those ages 50–64 every 5 years.

Canada

In Canada, an estimated 1,300 women will have been diagnosed with cervical cancer in 2008 and 380 will have died.

Australia

Australia had 734 cases of cervical cancer (2005). The number of women diagnosed with cervical cancer has dropped on average by 4.5% each year since organised screening began in 1991 (1991–2005). Regular twice-yearly Pap tests can

reduce the incidence of cervical cancer up to 90% in Australia, and save 1,200 Australian women from dying from the disease each year.

India

In India, the number of people with cervical cancer is rising, but overall the age-adjusted rates are decreasing. Usage of condoms in the female population has improved the survival of women with cancers of the cervix.

4. MATERIALS AND METHODS

4.1. Preparation of the drug

Drug selection

In this dissertation of *"**Rasa Karpoora Kuligai**"* has been selected from the classical siddha literature,***"Gunapadam Thathu Jeeva Vaguppu.***.

Ingredients of the drug are,

1. Purified Pooram (Calomel/Hydrargyrum subchloride)	-	21gm
2. Betel Leaves (Piper betle)	-	168gm
3. Pepper (Piper nigrum)	-	126gm
4. Garlic (Allium sativum)	-	84gm
5. Betel leaf juice	-	q/s
6. mother s milk	-	q/s

Collection of the drugs:

All the raw materials were obtained from country drug shop, Tirunelveli and Nagercoil districts.

Identification and Authentication:

All raw drugs were identified and Authenticated by the experts of Gunapadam *(pharmacology)* and Medicinal Botany departments, in Government Siddha Medical College Palayamkottai, Tirunelveli.

The specimen samples of the identified raw drugs were presented in the laboratory of PG Gunapadam for future references.

Purification of raw drugs:

Calomel:

The poultice made of betel leaf and pepper each 8.75gm is taken and dissolved in 1.3 litre of water. Calomel 35gm tied with a cloth and immersed in the liquid from the cross bar without touching the bottom of the vessels and heated. After the water is reduced to ¾ of its volume, the calomel is taken out, washed with water and dried to get it in purified form.

Pepper:

Soak pepper in the juice of Utthamani and dry it in sunlight till the juice dries up compeletely.

Garlic:

Peel the skin of garlic.

Betel Leaves:

Remove the stalk and midrib of betel leaves.

PREPARATIONS OF THE DRUG

(*Rasa Karpoora Kuligai*)

Ingredients of the drug are,

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2. Betel Leaves (Piper betle)	-	168gm
3. Pepper (Piper nigrum)	-	126gm
4. Garlic (Allium sativum)	-	84gm
5. Betel leaf juice	-	q/s

Process:

Calomel 21gm is soaked in mother's milk for three days. Garlic 84gm, pepper 126gm, and betel leaves 168gm are triturated with betel leaves juice for 15 hours (5 samam) and then rolled into pills like a unarmed night shade (sundai). Then it is dried in shade and preserve get the kuligai for further use.

Preservation of drug: It is stored in an air tight glass container.

Administration of the drug:

Form of the drug	:	Kuligai
Route	:	Enteral *(oral)*
Dosage	:	One pill twice a day for seven days
Adjuvant	:	Boiled and Cooled water.
Shelf Life	**:**	5 years

Indications:

- Scabies, syphilis, Cervical cancer, Penile cancer, Chronic ulcers and Pit wound.

4.2. PHARMACEUTICAL REVIEW

4.2.1. SIDDHA ASPECT OF THE FORMULATION

மாத்திரை:

மாத்திரை பாகம்:

கல்க திரவியங்களைத் தனியாகவேனும், பற்பசெந்தூரங்களுடன் சேர்த்தேனும் ஜலம், க'ாயம், மூலகைச்சாறு இவைகளுடன் ஏதேனும் ஒன்றில் நன்கு உறவாகும்படிஅரைத்து குறிப்பிட்ட அளவின்படி மாத்திரை செய்வதாகும்.

அரைப்பு விதி

மாத்திரை செய்வதற்காக நியமிக்கப்பட்ட கல்கங்களை தனித்தனியாகச் சூரணித்து அளவின்படி கல்வத்தில் இட்டு பசையுண்டாக்க கூடிய திரவியமொன்றினால் நனையும்படி பிசறிக் கல்வத்தின் கீழ்க்கையில் வைத்து கொண்டு கொஞ்சங் கொஞ்சமாகக் கல்வத்தின் மேல்கையில் ஏறும்படித் தள்ளி அரைக்க வேண்டும் இப்படி கீழ்க்கையில் இருந்த சரக்கையெல்லாம் மேலேறிய பின் மீண்டும் படிப்படியாக கீழ்கைக்குவரும்படி தள்ளிதள்ளி அரைத்தல் வேண்டும் இங்ஙனம் குறித்த காலம் வரையில் அரைப்பின் நிர்த்தோ'மான மாத்திரையாகும்.

மாத்திரை திரட்டும் பாகம்

அரைக்கும் போது, சரக்கு கல்வத்தில் சிறிதும் பற்றாமல் குழவியுடன் புரள வேண்டுவதுமன்றி அது குழவிக்கும் கல்வத்திற்கும் சம்பந்தமில்லாமல் இருக்க வேண்டுமட

வாசைனை திரவியங்கள் சேர்க்கும் காலம்

கஸ்தூரி, கோரோசனை, அம்பர், குங்குமப்பூ, பச்சைக் கற்பூரம், முதலானவைகளை மாத்திரை திரட்ட 1 நாழிகைக்கு முன் சேர்த்தரைப்பது நன்றுஅங்ஙன முடித்த மாத்திரைகளை நிழலுலர்த்தி உடனே கல்கார்க் சீசாவில்பதனஞ்செய்தல் வேண்டும்.

அரைப்பின் முன் பின் சேர்க்க வேண்டுவன

பிரயோகத்தில் அரைப்பதற்கு எது கடினமானதோ அதனை கல்வத்தில் இட்டு அரைத்து, அது பக்குவமானபின் மற்றவைகளைப் படிப்படியாக ஒன்றன் பின் ஒன்றாகச்சேர்த்து அரைத்தல் வேண்டும். வாளம் முதலிய பருப்பினங்கள் சேரும் பொழுதுமாத்திரை திரட்ட 1 சாமத்திற்கு முன் சேர்த்தல் வேண்டும். ஏனெனில் தாளகத்தைபோன்ற கடின சரக்குடன் மத்திமமான சரக்கு
சம்பந்தப்படின் தாளகம் எளிதில்அரைபடாது.

வாளம் முதலயவை நீண்ட அரைப்பிற்கு பின் மாத்திரை செய்து வரும்போது, அதனிலிருந்து தைலம் வெளிப்படும் அதனால் அதன் வன்மை குறையும்

4.2.2. MODERN ASPECT OF THE FORMULATION

Tablet (Pill)-*Kuligai*

A tablet is a pharmaceutical dosage form it otherwise called as caplet. Medicinal tablets are called as "pills". Originally "pills" referred specifically to a soft mass rolled into a ball shape, rather than a compressed powder. (wikipedia. org).

As per Indian Pharmacopeia 2007 defined the Tablets are solid dosage forms each containing a unit dose of one or more medicaments. They are anticipated for oral route. A tablet consists an active medicament with excipients which are in powder form are compressed or pressed into a solid dosage form. About two third drugs prescribed are in solid dosage form and tablets include half of them.

Classification:

As per IP2007 tablets are majorly classified into following categories (Indian pharmacopoeia 2007)

1. Uncoated Tablets:

This type of tablets contains single layer or more than one layer tablet consisting of active ingredient with the excipients, no additional cover is applied on to it after the compression.

2. Coated Tablets:

Coated types of tablets have an additional coating layer on it after the tablet was compressed, the coating layer of tablets formed with sugar, gums, resins, inactive or insoluble fillers, plasticisers, polyhydric alcohols, waxes.

3. Dispersible Tablets:

These are the film coated or uncoated tablets because a uniform dispersion when suspended in water

4. Effervescent Tablets:

These type of tablets which are uncoated and are planned to be dissolved and produce an dispersion before they are administered the dissolution is achieved by the reaction between an organic acid and bicarbonate which produce CO2, thus produced CO2 will disintegrate the tablet so which dissolves in the solution to produce an suspension which was rapidly absorbed.

5. Modified-release Tablets:

These types of tablets are the coated or uncoated tablets which are designed in such a way that the rate or location of the active ingredient released is modified. It includes enteric coated tablets, prolong release tablet or delay release tablet.

A) Enteric-coated Tablets:

These are also called as gastro resistant tablets as they resistant to the gastric juices; these are formulated by coating the tablet with anionic polymer of methylacrylic acid and their esters or by coating with cellulose acetyl pthylate.

Ex: erythromycin, NSAIDS

B) Prolonged- release Tablets:

These types are otherwise called as sustain release tablets or extended release tablets was formulated in such a way that the active ingredient is released for a prolong duration of time and is available in systemic circulation after administration.

C) Delayed-release Tablets:

This dosage form was planned to release the drug after some time delay or after the tablet has passed one part of the GIT into another. All enteric coated tablets are type of delayed action tablet but all delayed action of tablets was not enteric or not intended to produce enteric action.

6. Soluble Tablets:

These are coated or uncoated tablets which are planned to dissolve in water before they are administered.

7. Tablets for Use in the Mouth:

These are the tablet formulations which are planned to be show local action in the buccal cavity. These include buccal tablet, Sublingual Tablets and Troche or lozenges.Buccal tablets are placed in between the cheek and gingival. Sublingual tablets are placed below the tongue Eg: glyceryl trinitrate.

8. Tablets for other routes of administration:

These include implantable tablets and vaginal tablet. These are inserted in to the rectum or vagina for their local or systemic action.

4.2.3.ADVERSE EFFECT OF CHEMOTHERAPHY

CHEMOTHERAPHY DRUGS USED IN GYNAECOLOGIGIC CANCER

Cycle Non Specific Agents :

Alkylating Agents : Alkylating agents prevent cell division primarily by cross linking strands of DNA. Because of continued synthesis of their cell constituents, such as RNA and Protein, growth is unbalanced and the cell dies. Activity of Alkylating agents does not depend on DNA synthesis in the target cells.

TABLE : 1

Drug	**Dose and Route of Administration**	**Acute side effects**	**Toxicity**
Cyclophosphamide	500-1500 mg/m^2 single dose IV	Nausea, Vomiting	Bone Marrow Depression Alopecia Cystitis
Chlorambucil (Leukeran)	0.1-0.2 mg/kg/day PO	Nausea, Vomiting	Bone Marrow Depression
Melphalan	0.2 mg/kg/day PO for 4 days every 4 - 6 weeks	Nausea, Vomiting	Bone Marrow Depression
Thiotepa	0.2 mg/kg/day for 5 days	None	Bone Marrow Depression
Ifosfamide	7-10g/m^2 IV over 3-5 daysevery 3 - 4 weeks	Nausea, Vomiting	Bone Marrow Depression Alopecia Cystitis

Cycle Specific Agents

Antimetabolites : Antimetabolites act by inhibiting essential metabolic processes that are required for DNA and RNA synthesis.

TABLE : 2

Drug	**Dose and Route of Administration**	**Acute side effects**	**Toxicity**
5-Fluorouracil (5-Fu)	12mg/kg/day IV for 4 days	Occasional Nausea, Vomiting	Bone Marrow Depression Diarrhoea, Stomatitis Alopecia
Methotrexate	1.5 mg/kg IV for 5 days	None	Bone Marrow Depression Megaloblastic Anaemia, Diarrhoea, Stomatitis Alopecia, Hepatic fibrosis, Vasculitis, Pulmonary fibrosis
Cytarabine	200mg/m^2 daily for 5 days by continuous infusion	Nausea, Vomiting	Bone Marrow Depression Megaloblastosis, Leucopenia, Thrombocytopenia
Gemcitabine	800-1000mg/m^2 IV weekly every 3 weeks	Mild Nausea, Vomiting Malaise, Maculopopular Rash	Bone Marrow Depression

Antibiotics :

Several cytotoxic Antibiotic have come into use for chemotherapy of certain neoplasms. Those used in gynaecological cancers are given in table.

TABLE : 3

Drug	**Dose and Route of Administration**	**Acute side effects**	**Toxicity**
Actinomycin D (Dactinomycin)	15mg/kg/day IV	Pain on focal infiltration with skin Necrosis, Nausea and Vomiting, Cramps and Diarrhoea	Bone Marrow Depression Stomatitis, Erythema, Hyperpigmentation and Desquamation in area of Previous irritation
Mitomycin C	0.05 mg/kg/day IV for 6 days then alternate days until a 50 mg total dose	Nausea and Vomiting Local inflammation and Ulceration if extra vasated	Neutropenia, Thrombocytopenia, Oral Ulceration, Diarrhoea
Bleomycin	10-20mg/m^2 IV	Fever Chills, Nausea, Vomiting, Local pain and Phlebitis	Hyperpigmentation of skin, Pal Phemonitis with dyspnoea, fibrosis, especially with more than 400mg total dose
Doxorubicin (Adriamycin)	60-100 mg/m^2 IV every 3 weeks	Nausea, Vomiting, Fever local Phlebitis, Necrosis if extra vasated, red urine	Bone Marrow Depression, Alopecia, Cardiac Toxicity, related to Cumulative dose Atrophy of myocardia if a total dose of 450-500 kg/mg is exceeded

Plant Alkaloids :

The plant alkaloids arrest cells in Metaphase by binding the microtubular protein used in the formation of the mitotic spindle.

TABLE : 4

Drug	**Dose and Route of Administration**	**Acute side effects**	**Toxicity**
Vinblastine (Velban)	0.10-0.15 mg/kg/1 week IV	Severe prolonged inflammation if extra vasated, Nausea, Vomiting, Headache, Parasthesias	Bone Marrow Depression Alopecia, Muscle Weakness, Pheripheral Neuropathy, Mental depression and Stomatitis
Vincristine (Oncovin)	0.4-1.4 mg/m^2 IV weekly	Local inflammation if extra vasated	Parasthesias, Weakness loss of reflexes, Constipation hoarseness, foot drop, Marrow toxicity, anaemia, alopecia
Etoposide (VP-16)	100 mg/m^2 IV day 1:3:5 repeat in 4 weeks	Nausea and Vomiting	Leucopenia, Thrombocytopenia, Alopecia, Headache, Fever, Occasional Hypotension
Pacilitaxel (Taxol)	170-250 mg/m^2 IV every 3 - 4 weeks	Allergic reaction Nausea, Vomiting	Bone Marrow Depression Severe allergic like reactions with facial erythema, dyspnoea, Tachycardia, Hypotension, Cardiotoxicity, Alopecia, Stomatitis, Fatique

Miscellaneous Chemotherapy Drugs :

Certain Neoplastic agents are available that do not clearly fit into any of the above categories.

TABLE : 5

Drug	**Dose and Route of Administration**	**Acute side effects**	**Toxicity**
Cisplantin (Cisdiamminedichloro Platinum)	50-100 mg/m^2 IV every 3 weeks	Nausea and Vomiting	Renal damage, Moderate myelosuppresion, Neurotoxicity, severe renal damage can be avoided by not exceeding a total dose of 500 mg/m^2 in any treatment course
Carboplatin (Paraplatin)	250-400 mg/m^2 IV by 24 hr. continuous infusion every 2-4 weeks	Mild Nausea and Vomiting	Bone Marrow Depression esp. Thrombocytopenia
Hydroxyurea	80mg/kg PO every 3 days	Anorexia and Nausea	Bone Marrow Depression MegaloblasticAnaemia, Stomatitis, Diarrhoea, Alopecia
Hexamethyl Melamine	4-12 mg/kg/day PO in divided doses	Nausea and Vomiting	Bone Marrow Depression Neurotoxicity both central and Peripheral
Hycomptamine (Topotecan)	1.5 mg/m^2 daily for 5 days	Maculopopular Pruritic exanthema	Bone Marrow Depression

Medroxy Progesterone Acetate (MPA)	400-800 mg/week IM	None	Liver function Abnormalities alopecia and hypersensitivity reactions
Tamoxifen	10-20 mg PO twice daily	Nausea	Hot flashes, Pruritis Vulvae, Occasional Vaginal bleeding
Leuprolide	1mg/day Subcutaneously	Nausea	Hot flashes

DRUG TOXICITY :

The mechanism of toxicity is similar to the one producing the desired cytotoxic effect chemotherapeutic agents can damage even organs with limited cell proliferation. Chemotherapeutic agents must be used at doses that produce some degree of Toxicity to normal tissue in orders to be effective.

HAEMATOLOGIC TOXICITY :

The most common haematologic adverse effects include myelosuppression, manifested primarily as Leucopenia, thrombocytopenia and anaemia. The majority of chemotherapeutic agents produce myelosuppression. Most of these produce a maximum drop in leucocyte and platelet counts between day 7 and day 14 following drug administration, with recovery completed between day 21 and day 28.

DERMATOLOGIC TOXICITY :

EXTRAVASION INJURY :

The administration of chemotherapy generally involves the use of veins located between the dermis and the subcutaneous fat of the upper extremity. Extravasion of certain cytotoxic drugs into this space can cause a full thickness of skin and in areas with little subcutaneous fat, damage to Nerves, tendons and Muscles as well.

RADIATION ENHANCEMENT EFFECTS :

Certain cytotoxic drugs have the capacity to cause damage to previously irradiated tissue. Particularly skin and mucous membrane. Drugs commonly associated with this type of reaction include Actinomycin D, Doxorubicin, 5-Fluorouracil, and Hydroxyurea. The reaction assumes the form of an erythema

followed by dry desquamation with occasional ulceration. Management ranges from topical steroids and cool soaks for mild to moderate reactions, to surgical debridement for more severe reactions associated with ulceration.

ALOPECIA :

Hair loss associated with chemotherapy is a result of the effect of cyrotoxic agents on the rapidly proliferating tissue of the hair follicle. Inhibition of the activities of the growth cycle results in a failure to maintain the size of the root and the diameter of the shaft of the hair. The resultant thinner shaft is more susceptible to breakage with consequent loss of hair.

GASTRO INTESTIONAL EFFECTS :

Nausea and Vomiting secondary to chemotherapy are mediated through the vomiting centre in the medulla stimulation of this center arises from the chemoreceptor trigger zone, which lies close to the vomiting centre, (or) from stimuli transmitted from the cerebral cortex, gastrointestinal tract, heart and vestibular apparatus.

MUCOSITIS :

The term mucositis refers to the effect of certain chemotherapeutic agents on the epithelial lining of the gastrointestinal tract and includes stomatitis, cheilosis, glossitis, esophagitis and oral ulceration.

DIARRHOEA AND CONSTIPATION :

Diarrhoea, particularly bloody diarrhoea may be associated with those agents that produce mucositis, and is usually associated with severe mucositis in other portion of the gastrointestinal tract. In patients with protracted diarrhoea, the possibility of pseudomembranous colitis need to be ruled out.

Constipation is most often seen in patients receiving vinca alkaloids, particularly vincristine as a consequence of automatic neuropathy. The problem usually occurs within 3 days of drug administration. Management is conservative, with resolution expected over a 2 week period.

HEPATOTOXICITY :

Hepatotoxicity is uncommon mild elevations in transaminase alkaline phophatase and bilirubin are seen with many agents, but rarely the condition is severe. The management of all these reported hepatotoxicities requires the cessation of the affording agent when the liver abnormality is detected.

CARDIAC EFFECTS :

The principal chemotherapeutic agents associated with cardiotoxic effects are the anthracyclineantitumour antibiotics. i.e. daunrubicine and doxorubicin. The arrghythmias and electro cardiographic changes may occur during or immediately after drug administration. Cardiomyopathy is directly related to the cumulative total drug dose with doxorubicin at total doses higher than 550 mg/m^2, the incidence of cardiomyopathy associated with intractable congestive heart failure reaches 30%.

PULMONARY EFFECTS :

Pulmonary toxicity secondary to chemotherapy takes the form of a diffuse interstitial pneumonitis, which may eventually develop into pulmonary fibrosis. The drug first associated with these complications is bleomycin, onset is heralded by a dry cough dysphoea and fine bibasilar rales. Other drugs associated with a similar picture are Mitomycin C, the nitrosoureas, busulfan, and Methotrexate.

GENITOURINARY EFFECTS :

RENAL EFFECTS :

The drugs that most commonly produce direct renal toxicity include cisplantin, mitomycin C, mithramycin, methotrexate, and the nitrosources. The most prominent of these agents producing renal toxicity is cisplantin. The frequency of nephrotoxicity can be reduced by the induction and continuation of a urine flow in excess of 100ml/hr. prior to, during and for several hours after the drug administration.

BLADDER EFFECTS :

Significant toxicity to the lining of the urinary bladder has been reported with cyclophosphamide and the related compound ifosfamide. The urotoxicity of ifostamide, characterized by haemorrhagic cystitis, is due to the toxic metabolites 4 hydroxyifosfamide and acrolein.

GONADAL EFFECTS :

There have been no large scale detailed studies of drug effects on gonodal function. The reported effects are testicular atrophy, failure of spermatogenesis, and amenorrhoea. These agents mustard type alkylating agents, busulfan, the vinca alkaloids belomycin, hydroxyurea and procarbazine.

NEUROLOGICAL EFFECTS :

Acute encephalopathic syndrome have been reported with a variety of different chemotherapeutic agents. Management requires the cessation of the drug

and supportive care of the patient, pheripheral neuropathies are generally manifestated by both sensory and motor components, and are dose related. Pheripheral neuropathy is the principal non-haematologic dose limiting side effect of paclitaxel. The incidence of neuropathy is dose depent. Amifostine appears to have a neuroprotective activity.

EVALUATION OF NEW AGENTS :

A number of trials are necessary to demonstrate the use of a new agent in regular medical practice. These trials are define as follows :

Phase 1 :

These trials are designed to test new drugs at various doses to evaluate toxicity and determine the tolerance to a particular agent.

Phase 2 :

These attempt to determine the therapeutic effectiveness as well as the extent of toxicity of the particular agent at doses expected to be effective against specific tumour types.

Phase 3 :

These are designed to compare the drug to treatment currently in use. They are prospective randomised double blind trails.

4.3. STANDARDIZATION OF DRUG:

The standardization of drug is essential to exhibit the purity and quality and quantity of drug. This is basically done by biochemical, physiochemical, phytochemical and instrumental analysis.

The physiochemical analysis have been done at Aravindh herbal labs (p) ltd, Rajapalayam, Biochemical analysis were done at Govt Siddha Medical College Palayamkottai and microbiological analysis were done at inbiotics, William hospitial campus ms road, Nagerkovil,And the instrumental analysis was done at Indian Institute of Technology (IIT),Chennai-36 .

Organoleptic character

The organoleptic characters of the sample drug were evaluated.1gm of the test drug was taken and the colour, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result is noted

4.3.1. PHYSICO CHEMICAL ANALYSIS

Physicochemical studies of the trial drug have been done according to the WHO guidelines.

Determination of Ash Values:

Total Ash:

3g is accurately weighed and incinerated in a crucible dish at a temperature not exceed 450^{o}C until free from carbon. It is then cooled and weighed. The % w/w of ash with reference to the air-dried powder is calculated

Water Soluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by substracting the weight of insoluble ash from the weight of total ash.

Acid insoluble Ash:

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained.

Determination of Extractive Value:

Alcohol Soluble Extractive Value:

3g of test drug powder is weighed and macerated with 100ml of ethanol in a closed container for 24 hours. The resulting solution is shaken continuously for 6 hours and allowed to stand and soak for 18 hours. The solution is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

Water soluble Extractive value:

3g of test drug powder is weighed and macerated with chloroform and water, respectively, at 80^{o}C for 24 hrs. The resulting solution is shaken continuously for 6

hours and allowed to stand and soak for 24 hrs then filtered. The solution from both chloroform and water respectively is filtered and evaporated of the filtrate in a flat bottomed shallow dish and dried at 105^{o}C then cooled and weighed.

Loss on Drying:

The powdered drug is dried in the oven at 100- 105°C to constant weight. The result was noted.

THIN LAYER CHROMATOGRAPHY

Thin-layer chromatography(TLC) is a chromatographic technique that is useful for separating organic compounds. Because of the simplicity and rapidity of TLC, it is often used to monitor the progress of organic reactions and to check the purity of products.TLC is a simple, quick and inexpensive procedure that gives how many components are in a mixture.TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound(preferably both run on the same TLC plate).Chromatography works on the principle that different compounds will have different solubilities and adsorbtion to the two phases between which they are to be partitioned. As the solvent rises by capillary action up through the adsorbent, differential partitioning occurs between the components of the mixture dissolved in the solvent stationary adsorbent phase. The more strongly a given component of a mixture is adsorbed on to the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate.

The following are some common uses of Thin-Layer Chromatography

1. To determine the number of components in a mixture.
2. To determine the identity of two substances.
3. To monitor the progress of a reaction.
4. To determine the effectiveness of a purification.

Apparatus

Flat glass plates of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions and which allow accommodation of the specified migration path-length. The plates are prepared as described below; alternatively,

commercially prepared plates may be used.An aligning tray or a flat surface on which the plates can be aligned and rested when the coating substance is applied.

The adsorbent or coating substance consisting of finely divided adsorbent materials, normally 5 µm to 40 µm in diameter, is suitable for chromatography. It can be applied directly to the plate or can be bonded to the plate by means of Plaster of Paris (Hydrated Calcium Sulphate) or with any other suitable binders. The adsorbent may contain fluorescing material to help in visualising spots that absorb ultra-violet light.A spreader which, when moved over the glass plate, will apply a uniform layer of adsorbent of desired thickness over the entire surface of the plate.A storage rack to support the plates during drying and transportation.

A developing chamber that can accommodate one or more plates and can be properly closed and sealed. The chamber is fitted with a plate support rack that supports the plates, back to back, with lid of the chamber in place.Graduated micro-pipettes capable of delivering microlitre quantities say 10 µl and less.

A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Preparation of plates:

Unless otherwise specified in the monograph, the plates are prepared in the following manner. Prepare a suspension of the coating substance in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, spread a uniform layer of the suspension, 0.25 to 0.30 mm thick, on a flat glass plate 20 cm long. Allow the coated plates to dry in air, heat at 100º to 105º for at least 1 hour (except in the case of plates prepared with cellulose when heating for 10 minutes is normally sufficient) and allow to cool, protected from moisture. Store the plates protected from moisture and use within 3 days of preparation. At the time of use, dry the plates again, if necessary, as prescribed in the monographs

Method

Unless unsaturated conditions are prescribed, prepare the tank by lining the walls with sheets of filter paper; pour into the tank, saturating the filter paper in the process, sufficient of themobile phase to form a layer of solvent 5 to 10 mm deep, close the tank and allow to stand for 1 hour at room temperature. Remove a narrow strip of the coating substance, about 5 mm wide, from the vertical sides of the plate.

Apply the solutions being examined in the form of circular spots about 2 to 6 mm in diameter, or in the form of bands (10 to 20 mm x 2 to 6 mm unless otherwise specified) on a line parallel with, and 20 mm from, one end of the plate, and not nearer than 20 mm to the sides; the spots should be 15 mm apart. If necessary, the solutions may be applied in portions, drying between applications. Mark the sides of the plate 15 cm, or the distance specified in the monograph, from the starting line. Allow the solvent to evaporate and place the plate in the tank, ensuring that it is as nearly vertical as possible and that the spots or bands are above the level of the mobile phase. Close the tank and allow to stand at room temperature, until the mobile phase has ascended to the marked line. Remove the plate and dry and visualise as directed in the monograph; where a spraying technique is prescribed it is essential that the reagent be evenly applied as a fine spray.

For two-dimensional chromatography dry the plate after the first development and carry out the second development in a direction perpendicular to the first.

When the method prescribed in the monograph specified 'protected from light' or 'in subdued light' it is intended that the entire procedure is carried out under these conditions.

Visualisation

The phrases ultra-violet light (254 nm) and ultra-violet light (365 nm) indicate that the plate should be examined under an ultra-violet light having a maximum output at about 254 or at about 365 nm, as the case may be.

The term secondary spot means any spot other than the principal spot. Similarly, a secondary band is any band other than the principal band.

Rf. Value

Measure and record the distance of each spot from the point of its application and calculate the Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

MICROBIAL LIMIT TESTS:

DETERMINATION OF TOTAL AEROBIC MICROBIAL COUNT:

Dissolve 10 g or dilute 10 ml of the preparation being examined, unless otherwise specified, in buffered sodium chloride-peptone solution Ph 7.0 or any other suitable medium shown to have no antimicrobial activity under the conditions of test and adjust the volume to 100 ml with the same medium. If necessary, adjust the pH to about 7.

Membrane filtration:

Use membrane filters 50 mm in diameter and having anominal pore size not greater than 0.45 μm the effectiveness of which in retainingbacteria has been established for the type of preparation being examined. Sterilise andassemble the filtration apparatus described under tests for sterility.

Transfer 10 ml or a quantity of each dilution containing 1 g of the preparation being examined to each of two membrane filters and filter immediately. If necessary, dilute the pretreated preparation so that a colony count of 10 to 100 may be expected. Wash each membrane by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered sodium chloride-peptone solution pH 7.0. For fatty substances add to the liquid polysorbate 20 or polysorbate 80. Transfer one of the membrane filters, intended for the enumeration of bacteria, to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, to the surface of a plate of Sabouraud dextrose agar with antibiotics.

Incubate the plates for 5 days, unless a more reliable count is obtained in shorter time, at 30º to 35º in the test for bacteria and 20º to 25º in the test for fungi. Count the number of colonies that are formed. Calculate the number of micro-organisms per g or per ml of the preparation being examined, if necessary counting bacteria and fungi separately.

Plate count: For bacteria:

Using Petri dishes 9 to 10 cm in diameter, add to each dish a mixture of 1 ml of the pretreated preparation and about 15 ml of liquefied casein soyabean digest agar at not more than 45º. Alternatively, spread the pretreated preparation on the surface of the solidified medium in a Petri dish of the same diameter. If necessary, dilute the pretreated preparation as described above so that a colony count of not more than 300

may be expected. Prepare at least two such Petri dishes using the same dilution and incubate at 30º to 35º for 5 days, unless a more reliable count
is obtained in a shorter time. Count the number of colonies that are formed. Calculate the results using plates with the greatest number of colonies but taking 300 colonies per plate as the maximum consistent with good evaluation.

For fungi:

Proceed as described in the test for bacteria but use Sabouraud dextrose agar with antibiotics in place of casein soyabean digest agar and incubate the plates at 20º to 25º for 5 days, unless a more reliable count is obtained in a shorter time. Calculate the results using plates with not more than 100 colonies.

TESTS FOR SPECIFIED MICRO-ORGANISMS:

Pretreatment of the sample being examined – Proceed as described under the test for total aerobic microbial count but using lactose broth or any other suitable medium shown to have no antimicrobial activity under the conditions of test in place of buffered sodium chloride-peptone solution pH 7.0.

ESCHERICHIA COLI:

Place the prescribed quantity in a sterile screw-capped container, add 50 ml of nutrient broth, shake, allow to stand for 1 hour (4 hours for gelatin) and shake again. Loosen the cap and incubate at 37º for 18 to 24 hours.

Primary test:

Add 1.0 ml of the enrichment culture to a tube containing 5 ml of MacConkey broth. Incubate in a water-bath at 36º to 38º for 48 hours. If the contents of the tube show acid and gas carry out the secondary test.

Secondary test:

Add 0.1 ml of the contents of the tubes containing (a) 5 ml of MacConkey broth, and (b) 5 ml of peptone water. Incubate in a water-bath at 43.5º to 44.5º for 24 hours and examine tube (a) for acid and gas and tube (b) for indole. To test for indole, add 0.5 ml of Kovac's reagent, shake well and allow to stand for 1 minute; if a red colour is produced in the reagent layer indole is present. The presence of acid and gas and of indole in the secondary test indicates the presence of Escherichia coli.

Carry out a control test by repeating the primary and secondary tests adding 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Escherichia coli (NCTC 9002) organisms, prepared from a 24-hour culture in nutrient

broth, to 5 ml of MacConkey broth. The test is not valid unless the results indicate that the control contains Escherichia coli.

SALMONELLA :

Transfer a quantity of the pretreated preparation being examined containing 1 g or 1 ml of the product to 100 ml of nutrient broth in a sterile screwcapped jar, shake, allow to stand for 4 hours and shake again. Loosen the cap and incubate at 35º to 37º for 24 hours.

Primary test:

Add 1.0 ml of the enrichment culture to each of the two tubes containing (a) 10 ml of selenite F broth and (b) tetrathionate-bile-brilliant green broth and incubate at 36º to 38º for 48 hours. From each of these two cultures subculture on at least two of the following four agar media: bismuth sulphate agar, brilliant green agar, desoxycholatecitrate agar and xylose-lysine-desoxycholate agar. Incubate the plates at 36º to 38º for 18 to 24 hours. Upon examination, if none of the colonies conforms to the description given in Table 2, the sample meets the requirements of the test for the absence of the genus Salmonella.

If any colonies conforming to the description in Table 2 are produced, carry out the secondary test.

Secondary test:

Subculture any colonies showing the characteristics given in Table 2 in triple sugar-iron agar by first inoculating the surface of the slope and then making a stab culture with the same inoculating needle, and at the same time inoculate a tube of urea broth. Incubate at 36º to 38º for 18 to 24 hours. The formation of acid and gas in the stab culture (with or without concomitant blackening) and the absence of acidity from the surface growth in the triple sugar iron agar, together with the absence of a red colour in the urea broth, indicates the presence of salmonellae. If acid but no gas is produced in the sub culture, the identity of the organisms should beconfirmed by agglutination tests.

Carry out the control test by repeating the primary and secondary tests using 1.0 ml of the enrichment culture and a volume of broth containing 10 to 50 Salmonella abony (NCTC 6017) organisms, prepared from a 24-hour culture in nutrient broth, for the inoculation of the tubes (a) and (b). The test is not valid unless the results indicate that the control contains Salmonella.

Table no: 6 – Test for Salmonella

Medium	**Description of colony**
Bismuth Sulphite agar	Black or green
Brilliant green agar	Small, transparent and colourless, or opaque, pinkish or white (frequently surrounded by a pink or red zone)
Deoxycholate – Citrate agar	Colourless and opaque, with or without black centers
Xylose-lysine-desoxy-cholate agar	Red with or without black centres

PSEUDOMONAS AERUGINOSA :

Pretreat the preparation being examined as described above and inoculate 100 ml of fluid soyabean-casein digest medium with a quantity of the solution, suspensionor emulsion thus obtained containing 1 g or 1 ml of the preparation being examined. Mix and incubate at 35º to 37º for 24 to 48 hours. Examine the medium for growth and if growth is present, streak a portion of the medium onthe surface of cetrimide agar medium, each plated on Petri dishes. Cover and incubate at 35º to 37º for 18 to 24 hours. If, upon examination, none of the plates contains colonies having the characteristics listed in Table 3 for the media used, the sample meets the requirement for freedom from Pseudomonas aeruginosa. If any colonies conforming to the description in Table 3 are produced, carry out the oxidase and pigment tests.

Streak representative suspect colonies from the agar surface of cetrimide agar on the surfaces of pseudomonas agar medium for detection of fluorescein and pseudomonas agar medium for detection of pyocyanin contained in Petri dishes. Cover and invert the inoculated media and incubate at 33º to 37º for not less than 3 days. Examine the streaked surfaces under ultra-violet light. Examine the plates to determine whether colonies conforming to the description in Table 3 are present.

If growth of suspect colonies occurs, place 2 or 3 drops of a freshly prepared 1% w/v solution of N,N,N1,N1-tetramethyl-4-phenylenediamine dihydrochloride on filter paper and smear with the colony; if there is no development of a pink colour, changing to purple, the sample meets the requirements of the test for the absence of Pseudomonas aeruginosa.

Table no: 7- Tests for Pseudomonas aeruginosa

Medium	**Characteristic colonical morphology**	**Fluorescence in UV light**	**Oxidase test**	**Gram stain**
Cetrimde agar	Generally greenish	Greenish	Positive	Negative rods
Pseudomonas agar medium for dedection of fluorescein	Generally colourless to yellowish	Yellowish	Positive	Negative rods
Pseudomonas agar medium for dedection of pyocyanin	Generally greenish	Blue	Positive	Negative rods

STAPHYLOCOCCUS AUREUS:

Proceed as described under Pseudomonas aeruginosa. If, upon examination of the incubated plates, none of them contains colonies having the characteristics listed in Table 4 for the media used, the sample meets the requirements for the absence of Staphylococcus aureus. If growth occurs, carry out the coagulase test. Transfer representative suspect colonies from the agar surface of any of the media listed in Table 4 to individual tubes, each containing 0.5 ml of mammalian, preferably rabbit or horse, plasma with or without additives. Incubate in water-bath at 37º examining the tubes at 3 hours and subsequently at suitable intervals up to 24 hours. If no coagulation in any degree is observed, the sample meets the requirements of the test for the absence of Staphylococcus aureus.

Table no:8 – Tests for Staphylococcus aureus

Selective medium	**Characteristic colonical morphology**	**Gram stain**
Vogel-Johnson agar	Black surrounded by yellow zones	Positive cocci (in clusters)
Mannitol-salt agar	Yellow colonies with yellow zones	Positive cocci (in clusters)
Baired-Parker agar	Black, shiny, surrounded by clear zones of 2 to 5 mm	Positive cocci (in clusters)

4.3.2. BIO CHEMICAL ANALYSIS:

Preliminary Basic and Acidic radical studies:

Preparation of the extract:

5gms of the test drug is weighed accurately and placed in a 250ml clean beaker. Then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

QUALITATIVE ANALYSIS FOR BASIC RADICALS:

Test for Calcium:

2ml of the above prepared extract is taken in a clean test tube. To this add 2ml of 4% Ammonium oxalate solution. Formation of white precipitate indicates the presence of calcium.

Test for Iron (Ferric):

The extract is acidified with glacial acetic acid and potassium ferro cyanide. Formation of blue colour indicates the presence of ferric iron.

Test for Iron (Ferrous):

The extract is treated with concentrated Nitric acid and ammonium thio-cyanate solution. Formation of blood red colour indicates the presence of ferrous iron.

Test for Zinc:

The extract is treated with potassium ferro-cyanide. Formation of white precipitate indicates the presence of zinc.

QUALITATIVE ANALYSIS FOR ACIDIC RADICALS:

Test for Sulphate:

2ml of extract is added to 5% barium chloride solution. Formation of white precipitate indicates the presence of sulphate.

Test for Chloride:

The extract is treated with silver nitrate solution. Formation of white precipitate indicates the presence of chloride.

Test for Phosphate:

The extract is treated with ammonium molybdate and concentrated nitric acid. Formation of yellow precipitate indicates the presence of phosphate.

Test for Carbonate:

On treating the extract with concentrated hydrochloric acid giving brisk effervescence indicates the presence of carbonate.

Test for starch:

The extract is added with weak iodine solution. Formation of blue colour indicates the presence of starch.

Test for albumin:

The extract is treated with Esbach`s reagent. Formation of yellow precipitate indicates the presence of albumin.

Test for tannic acid:

The extract is treated with ferric chloride. Formation of bluish black precipitate indicates the presence of tannic acid.

Test for unsaturation:

The extract is treated with potassium permanganate solution. The dis-colourization of potassium permanganate indicates the presence of unsaturated compounds.

Test for the reducing sugar:

5ml of Benedict`s qualitative solution is taken in a test tube and allowed to boil for 2 minutes and added 8-10 drops of the extract and again boil it for 2 minutes. Any colour change indicates the presence of reducing sugar.

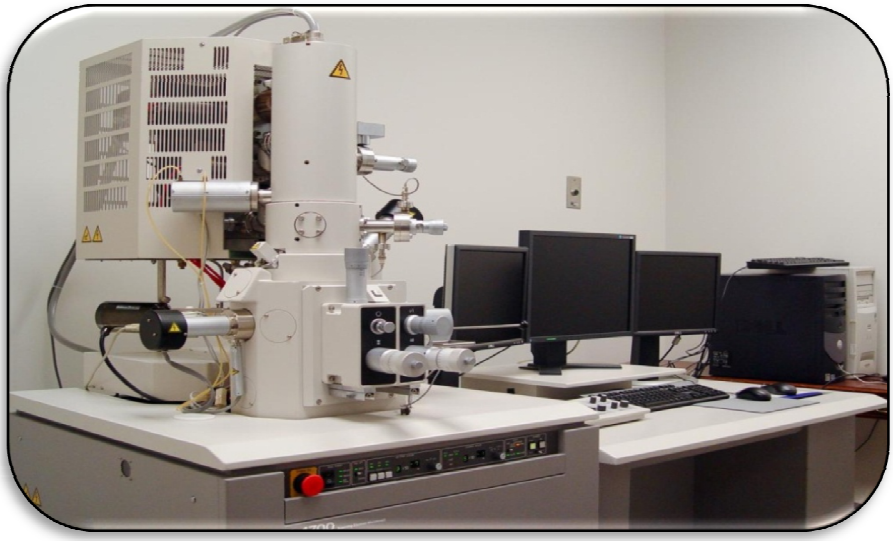
Test for amino acid:

One or two drops of the extract is placed on a filter paper and dried it well. After drying, 1% Ninhydrin is sprayed over the same and dried it well. Formation of violet colour indicates the presence of amino acid.

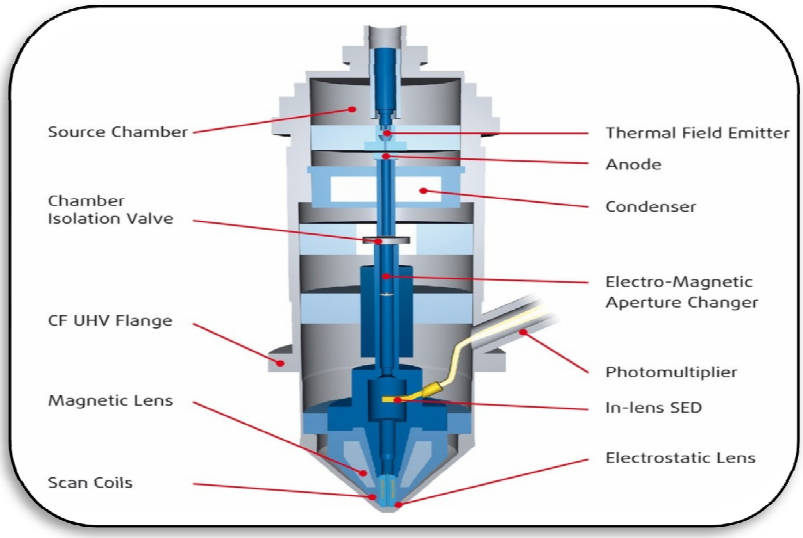
4.3.3. INSTRUMENTAL ANALYSI

SEM *(SCANNING ELECTRON MICROSCOPE)*

SEM INSTRUMENT



SEM - SCANNING ELECTRON MICROSCOPE



MECHANISM

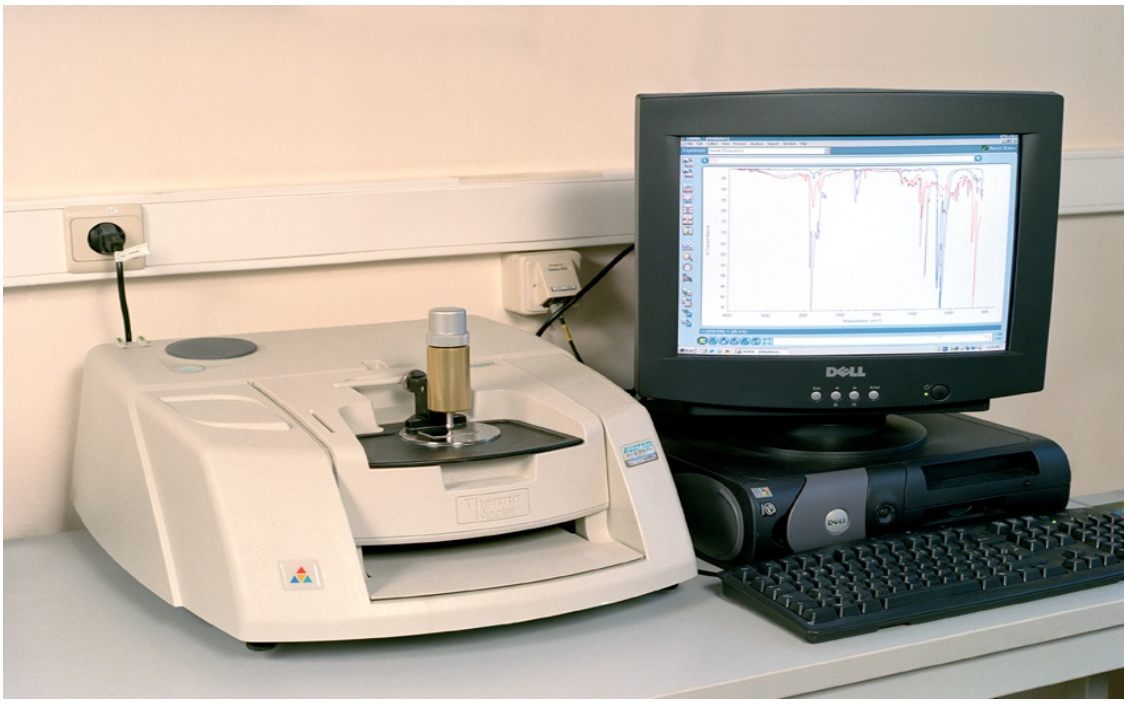
In scanning electron microscope high-energy electron beam is focused through a probe towards the sample material. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it is collected by a appropriate detector.

The types of signal produced by a scanning electron microscope include

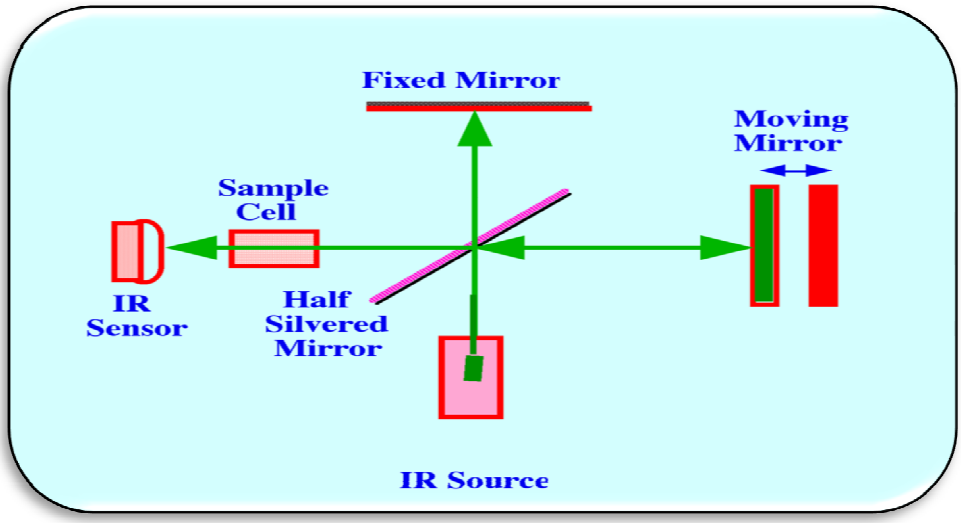
- Secondary electrons
- back scattered electrons
- characteristic x-rays, light
- specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

FT-IR(Fourier Transform Infrared Spectroscopy):



FTIR INSTRUMENT



FTIR MECHANISM

Model	**:**	**Spectrum one: FT-IR Spectrometer**
Scan Range	**:**	**MIR 450-4000 cm-1**
Resolution	**:**	**1.0 cm-1**
Sample required	**:**	**50 mg, solid or liquid.**

It is the preferred method of infrared spectroscopy. FT-IR is an important and more advanced technique. It is used to identify the functional group, to determine the quality and consistency of the sample material and can determine the amount of compounds present in the sample. It is an excellent tool for quantitative analysis.

In FT-IR infrared is passed from a source through a sample. This infrared is absorbed by the sample according to the chemical properties and some are transmitted. The spectrum that appears denotes the molecular absorption and transmission. It forms the molecular fingerprint of the sample. Like the finger print there is no two unique molecular structures producing the same infrared spectrum. It is recorded as the wavelength and the peaks seen in the spectrum indicates the amount of material present.

FT-IR is the most advanced and the major advantage is its

- Speed
- Sensitivity
- Mechanical simplicity
- Internally calibrated

ICP-OES *(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)*

ICP-OES *(INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)*

Manufacturer : Perkin Elmer

Model : Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer *(ICP)*

Principle :

An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which is a high temperature zone *(8,000– 10,000°C)*. The analysts are heated *(excited)*to different (atomic and/or ionic) states and produce characteristic optical emissions *(lights)*. These releases are separated based on their respective wavelengths and their strengths are measured *(spectrometry)*. The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification is an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of analysis where chemical speciation is relevant *(such as the concentration of ferrous iron or ferric iron)*, only total essential concentration is analysed by ICP-OES.

Application:

The analysis of major and minor elements in solution samples.

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms

Mechanism:

In plasma emission spectroscopy *(OES),* a sample solution is presented into the core of inductively coupled argon plasma *(ICP),* which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light is collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light is then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardizationvalues

The Inductively Coupled Plasma Optical Emission Spectrometric *(ICP-OES)* analysis was done in Saif, IIT Madras, and Chennai-36 using Perkin Elmer Optima 5300 DV.

Sample preparation:

Inductively Coupled Plasma Spectroscopy techniques are the so-called "wet" sampling methods whereby samples are introduced in liquid form for analysis.

100 mg “Rasa karpoora kuligai” was occupied in a clean, dry test tube. To this, 3 ml Nitric acid was added and mixed well and allowedfor few minutes untill the reactions were completed. And then, 25 ml of Refined water, was added to prepare digested solution.

4.4. TOXICOLOGICAL STUDIES

PRECLINICAL TOXICITY STUDIES OF *RASA KARPOORA KULIGAI* ON WISTAR ALBINO RATS

4.4.1. ACUTE TOXICITY STUDY IN FEMALE WISTER RATS TO EVALUATE TOXICITY PROFILE OF MKC

OBJECTIVES

The aim of this Study is to evaluate the toxicity of the test substance mavilingu kasaya chooranam, when administered orally to Female Wister Rats with different doses, so as to provide a rational base for the evaluation of the toxicological risk to man and indicate potential target organs.

Guidelines followed:

(a) OECD Guidelines No. 423,

Study Design and Controls:

1) Female Wister Rats in controlled age and body weight were selected.

2) ***The test drug RKK*** was administered at **5 mg/kg, 10 mg/kg,300 mg/kg, 1000 mg/kg, and 2000 mg/kg** body weight of animal as suspension along with water.

3) The results were recorded on day 0, with single oral dosing period of 14 days.

EXPERIMENTAL PROCEDURE

1. ANIMALS

1.1 Supply

A total of 15 Female Wister Rats with an approximate age of 6 weeks and purchased from M/s.Venkateshwara Enterprises Pvt. Ltd, Bangalore. On their arrival a sample of animals was chosen at random and weighed to ensure compliance with the age requested. The mean weights of Female Wister Rats were 100-150 g respectively. The animals were housed in metabolic cages (55 x 32.7 x 19 cm), with sawdust litter, in such a way that each cage contained a maximum of 3 animals of the same sex.

All animals underwent a period of 20 days of observation and acclimatization between the date of arrival and the start of treatment. During the course of this period, the animals were inspected by a veterinary surgeon to ensure that they fulfilled the health requirements necessary for initiation of the Study.

1.2. Housing

The Female Wister Rats were housed in metabolic cages (55 x 32.7 x 19 cm), placed on racks. From the week before initiation of the treatment, each cage contained a maximum of 3 rats of the same sex and treatment group.

Each cage was identified by a card, color coded according to the dose level. This card stated the cage number, number and sex of the animals it contained, Study number, test substance code, administration route, dose level and Study Director's name, date of the arrival of the animals and initiation of treatment.

The temperature and relative humidity were continuously monitored. Lighting was controlled to supply 12 hours of light (7:00 to 19:00 hours) and 12 hours of dark for each 24-hour period.

The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

2.DIET

All the rats had free access to a pelleted rat diet. The diet was analyzed by the manufacturer to check its composition and to detect possible contaminants.

2.1. Water

The water was offered ad libitum in bottles.

3. Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table no-9 Numbering and Identification

Group No	Animal Marking
1	Head
2	Body
3	Tail

Table no 10 Numbering and Identification

Cage No	Group No	Animal Marking	Sex
1	I	H,B,T	Female
2	II	H,B,T	Female
3	III	H,B,T	Female
4	IV	H,B,T	Female
5	V	H,B,T	Female

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the animals

3. ADMINISTRATION ROUTE AND PROCEDURE

The test substance was administered orally. The Female Wister Rats belonging to the control group were treated with the vehicle (Water) at the same administration volume as the rest of the treatment groups.

3.1. Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then drug was administered orally as single dose using a needle fitted onto a disposable syringe of approximate size at the following different doses.

Table no -11 Doses

GROUP	DOSE
GROUP	**DOSE**
Group-I	**5 mg/kg**
Group-II	**50 mg/kg**
Group-III	**300 mg/kg**
Group-IV	**1000 mg/kg**
Group-V	**2000 mg/kg**

The test item was administered as single dose. After single dose administration period, all animals were observed for 14days.

Dose Preparation

RASA KARPOORA KULIGAI was added in distilled water and completely dissolved to form oral for administration. The dose was prepared of a required concentration before dosing by dissolving, in distilled water. It was mixed well. The

preparation for different doses was vary in concentrations to allow a constant dosage volume.

3.2. Administration

The test item was administered orally to each Female Wister rats as single dose using a needle fitted onto a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg bodyweight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

3.3.Observation period

All animals were observed for any abnormal clinical signs and behavioral changes. The appearance, change and disappearance of these clinical signs, if any, were recorded for approximately 1.0, 3.0 and 4.0 hours post-dose on day of dosing and once daily thereafter for14 days. Animals in pain or showing severe signs of distress were humanely killed. The cageside observation was included changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc were observed. At the 14th day, sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli) was conducted. Auditory stimuli responses were measured by clicker sound from approximately 30 cm to the rats; visual stimuli response were measured with the help of shining pen light in the eye of rats and placing a blunt object near to the eye of rats. Response to proprioceptive stimuli was measured by placing anterior/dorsal surface of animals paw to the table edge. The responses of reactions for these three exercises were normal in animals belonging to both the controls as well as drug treatment dose groups.

3.4.Mortality and Morbidity

All animals were observed daily once for mortality and morbidity at approximately 1.0, 3.0 and 4.0 hours post dose on day of dosing and twice daily (morning and afternoon) thereafter for 14 days

4.4.2 Sub-Acute Toxicity Study in Wister rats to Evaluate Toxicity Profile of RASA KARPOORA KULIGAI

1. Objective

The objective of this '**Sub-Acute Toxicity Study of MKC ON Wister Rats**' was to assess the toxicological profile of the test item when treated as a single dose daily. Animals should be observed for 28 days after the drug administration. This study provides information on the possible health hazards likely to arise from exposure over a relatively limited period of time.

2. Test Guideline Followed

OECD 407 Method - Sub-Acute Toxic Class Method (Repeated Dose 28-Day Oral Toxicity Study in Rodents)

3. Test Item Detail

Name: **RASA KARPOORA KULIGAI**

4.Test System Detail

The study was conducted on 5 male 5 female Wister rats for each group. These animals were selected because of the recommended rodent species for oral studies as per followed guideline and availability of Animals 8-12 weeks old male and female rats were selected after physical and behavioral examination. The body weight range was fallen within ± 20% of the mean body weight at the time of Randomization and grouping. The rats were housed in standard laboratory condition in Polypropylene cages, provided with food and water *adlibitum* in the Animal at M/s. Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, government of India.

5. Acclimatization

The animals were selected after veterinary examination by the veterinarian. All the selected animals were kept under acclimatization for a week.

6.Randomization & grouping

One day before the initiation of treatment (days 0- last day of acclimatization), the selected animals were randomly grouped into three different groups containing minimum 5 male and 5 female animals per group.

7.Numbering and Identification

The animals were marked on body with picric acid solution prepared in water. The marking within the cage was as below.

Table no-11 Numbering and Identification

Group No (C0NCENTRATION/DOSE)	Animal Marking
1. CONTROL	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
2. LOW DOSE OF RKK 200mg/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
3. MIDDLEDOSE OF RKK 400mg/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)
4. HIGH DOSE OF RKK 600mg/kg	H,B,T,HB,NM (MALE) H,B,T,HB,NM (FEMALE)

Cage No	Group No C0NCENTRATION/DOSE	Animal Marking	Sex
1	1. CONTROL	H,B,T,HB,NM H,B,T,HB, NM	Male Female
2	2. LOW DOSE OF RKK 200mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
3	3. MIDDLEDOSE OF RKK 400mg/kg	H,B,T,HB,NM H,B,T,HB, NM	Male Female
4	4. HIGH DOSE OF RKK 600mg/kg	H,B,T,HB,NM H,B,T,HB ,NM	Male Female

The group no., cage no., sex of the animal and animal no. were identified as indicated below using cage label and body marking on the Above

8. Husbandry

8.1 Housing

The Wister rats were housed in standard polypropylene cages with stainless steel top grill. Paddy husk was used as bedding. The paddy husk was changed at least twice in a week. From the week before initiation of the treatment, each cage contained a maximum of 10 rat of the differnt sex and treatment group.

8.2 Environmental conditions

The animals were kept in a clean environment with 12 hour light and 12 hour dark cycles. The air was conditioned at 22±3^{0}C and the relative humidity was maintained between 30-70% with 100% exhaust facility. The cages corresponding to each experimental group were distributed on racks in such a manner that external factors, such as environmental conditions, were balanced as far as possible.

8.3 Feed & feeding schedule

'Sai Durga Animal Feed, Bangalore. Feed was provided *adlibitum throughout* the study period, except over night fasting (18-20 hours) prior to dose administration. After the substance has been administered, food was with held for a further 3-4 hours.

8.4 Water

The water was offered *adlibitum* in bottles. There was periodically analyzed to detect the presence of possible contaminants

8.5 Doses

The doses for the study were selected based on literature search and range finding study. Following the period of fasting, the animals were weighed and then extract was administered orally as single dose using a needle fitted on to a disposable syringe of approximate size at the following different doses.

Table no -12 Dose level

TEST GROUP	C0NCENTRATION/DOSE TO ANIMALS (ml/kg body-weight/day)	NUMBER OFANIMALS
Group-1	**1. CONTROL**	10 (5MALE and5 FEMALE)
Group-II	**2. LOW DOSE OF RKK 200mg/kg**	10(5MALE and 5 FEMALE)
Group-III	**3. MIDDLEDOSE OF RKK 400mg/kg**	10(5MALE and 5 FEMALE)
Group-IV	**4. HIGH DOSE OF RKK 600mg/kg**	10(5MALE and 5 FEMALE)

The test item was administered as single dose daily. After single dose administration period, all animals were observed for 28 days.

Dose Preparation

RKK was added in distilled water and completely dissolved for oral administration. The dose was prepared of a required concentration before dosing by dissolving **RKK** in distilled water. It was mixed well. The preparation for different doses was vary in concentrations to allow a constant dosage volume.

8.6 Administration

The test item was administered orally to each rat as single dose using a needle fitted on to a disposable syringe of appropriate size at the following different doses. The concentration was adjusted according to its body weight. The volume was not exceeding 10 ml/kg body weight. Variability in test volume was minimized by adjusting the concentration to ensure a constant volume at all dose levels.

9. OBSERVATIONS

These observations were also performed on week-ends. The observations included but were not limited to changes in skin and fur, in the eyes and mucous membranes, in the respiratory, circulatory, central nervous and autonomous systems, somatomotor activity and behavior.

9.1. Clinical signs of toxicity

All the rats were observed at least twice daily with the purpose of recording any symptoms of ill- health or behavioral changes. Clinical signs of toxicity daily for 28 days.

9.2. Food intake

Prior to the beginning of treatment, and daily, the food intake of each cage was recorded for period of 28 days and the mean weekly intake per rats was calculated.

9.3. Water intake

Water intake was checked by visual observation during the Study. In addition, the water consumption in each cage was measured daily for a period of 28 days.

9.4 Bodyweight:

The body weight of each rat was recorded one week before the start of treatment, and during the course of the treatment on the day of initial, 3rd, 7th, 10th, 14th, 17th, 20th, 24th and 28th days (day of sacrifice). The mean weights for the different groups and sexes were calculated from the individual weights.

Blood Collection Blood was collected through retro-orbital sinus from all the animals of different groups on 28th day. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. Animals were fasted over night prior to the blood collection.

LABORATORY STUDIES

During the 4th week of treatment, samples of blood were withdrawn from the orbital sinus of 6 rats from each group, under light ether anesthesia after fasting for 16 hours. The blood samples are used to evaluate Hematological parameters like RBC, WBC, and PLATELETS etc….. The collected blood samples also centrifuged 10000 rpm in 10 minutes to separate the serum. The separated serum used to evaluate biochemical parameters like SGOT, SGPT, ALP and BILIRUBIN etc……...

Hematology

The following hematological parameters were analysed using Autoanalyser

Hb	:	Haemoglobin (g %)
PCV	:	Packed Cell Volume
WBC	:	White Blood Corpuscles (x103/cmm)
RBC	:	Red Blood Corpuscles (x106/cmm)

Blood Platelet count (x103/cmm)

Differential WBC count:

N	:	Neutrophils (%)
L	:	Lymphocytes (%)
M	:	Monocytes (%)
E	:	Eosinophils (%)
RDW	:	Red Cell Distribution Width.
MPV	:	Mean Platelet Volume

Clinical Biochemistry:

The following clinical Bio parameters were analysed using Auto analyser

Total serum protein (g/dl)

ALT/SGPT	:	Alanine amino transferase (U/L)
AST/SGOT	:	Aspartate amino transferase (U/L)
ALP	:	Alkaline serum phosphatase (U/L)
CHL	:	Cholesterol (mg/dL)
HDL	:	High density lipoprotein
TG	:	Triglyceride

TERMINAL STUDIES

Sacrifice and macroscopic examination

On completion of the 4 weeks of treatment, 18 Wister rats were sacrificed by ether inhalation. A full autopsy was performed on all animals which included examination of the external surface of the body, all orifices, cranial, thoracic and abdominal cavities and their contents both *in situ* and after evisceration. As the number of animals exceeded the number that could be sacrificed in one day, the autopsies were carried out over three consecutive days at the end of the treatment period.

Organ weights:

After the macroscopic examination the following organs were weighed after separating the superficial fat: Brain, Heart, Spleen Kidneys, Testes, Liver, Lungs, pancreas and stomach

4.5. PHARMOCOLOGICAL STUDY

4.5.1. CYTOTOXIC PROPERTIES OF RASA KARPOORA KULIGAI AGAINST HELA CELL LINE

Project Title: *In vitro* apoptosis study of test drug *Rasa karpoora kuligai*.

Aim of the study: Determination of apoptotic properties of test drug in HeLa cell line by DAPI staining.

Samples: KR.2019

Materials and Methods

Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India, DAPI from Himedia Mumbai.

Cell lines and Culture medium

HeLa cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test samples

For cytotoxicity studies, weighed test drug was separately dissolved in DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by MTT Assay

Principle: The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium

bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used (Francis and Rita, 1986).

Procedure: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM containing 10% FBS. Later to each well of the 96 well microtitre plate, polymer were placed in triplicates, later 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 4 h, to each well 150µl of DMEM supplemented with 2% FBS was added to the wells The plate was then incubated at 37^o C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and plate was washed with PBS and then media containing MTT (5 mg/ml) was added each well. The plates were gently shaken and incubated for 3 h at 37^o C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The solubilized formazan was then transferred into clean plate and absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100 \right)$$

Determination of Apoptotic property by DAPI staining

To characterize cell-specific apoptotic process in HeLa cells, analysis of chromatin condensation and nuclear fragmentation was done by DAPI staining using fluorescence microscopy. After treatment of 70–80% confluent HELA cells with varying concentration of sample 1 for 24 hr, cells were quickly washed with ice-cold PBS and fixed in ice-chilled acetone: methanol (1:1) mixture for 10 min at 4^{o}C in the dark. The cells were washed twice with ice-cold PBS and then incubated for 30 min with the DNA-specific fluorochrome, DAPI. The excess DAPI was removed with ice-cold PBS wash, and the cells were observed and photographed using fluorescence microscope at 40Xmagnification.

4.5.2. ANTIOXIDANT ACTIVITY OF RASA KARPOORA KULIGAI IN VITRO METHODS

4.5.2.1 INVITRO ANTI OXIDANT ACTIVITY RASA KARPOORA KULIGAI ON DPPH RADICAL SCAVENGING ACTIVITY

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl)

INTRODUCTION

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al[2001].The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1,1-diphenyl-2-picryl hydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

DPPH + [H-A] →DPPH-H + (A)

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

PROCEDURE

Different volumes (1.25-10µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture incubated in dark condition at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was read at 517nm.3ml of DPPH was taken as control.

CALCULATION

$$\% \text{ inhibition} = \frac{control-test}{control} X100$$

4.5.2.2 FERRIC REDUCING ANTIOXIDANT POWER (FRAP) RADICAL SCAVENGING ACTIVITY ON RASA KARPOORA KULIGAI

MATERIALS AND METHODS

Reagent preparation.

Reagents included 300 mmol/ - liter acetate buffer, pH 3.6 (3.1 g C2H3NaO2r3H2O (Rie- del-de Haen, Germany) and 16 ml C2H4O2 (BDH Laboratory Supplies, England) per liter of buffer solution); 10 mmol/liter TPTZ (2,4,6-tripyridyl-s-triazine,Fluka Chemicals, Switzerland) in 40 mmol/liter (BDH); 20 mmol/liter FeCl3r6H2O (BDH). Working FRAP reagent was prepared as required by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml FeCl3r6H2O solution.

Antioxidants.

Solid L-(/)-ascorbic acid extra pure crystals (Merck, Germany), uric acid, solid (BDH), al bumin, solid (bovine serum albumin, fraction V, Sigma), bilirubin calibrator solution (Sigma), and Trolox (Aldrich Chemical Co., U.S.A.) were used to prepare aqueous antioxidant solutions. DL-a-Tocopherol (Merck) was diluted in ethanol (Merck)

Reagent kits (Hoffman–LaRoche Ltd., Switzerland).Uric acid was measured by an enzymatic method, bili- rubin was measured by an alkaline picrate method and albumin was measured by a bromocresol green dye-binding method. These tests were performed on a CobasFara centrifugal analyzer (Roche) following the manufacturer's instructions and using commercially available calibrators and quality control samples.

Procedure

The FRASC assay was performed as described in detail elsewhere (Benzie & Strain, 1997, 1999). In brief, reductants ('antioxidants') in the sample reduce a ferric–tripyridyltriazine complex, present in stoichiometric excess, to the blue coloured ferrous form. The change of absorbance at 593 nm over 4 min is proportional to the combined (total) FRAP value of the antioxidants in the sample. In the FRASC assay, AA(Ascorbic Acid) is selectively destroyed by the addition of ascorbate oxidase to one of a pair of sample aliquots. In this case the absorbance change of a sample to which ascorbate oxidase was added is subtracted from the absorbance of a matching aliquot to which water, rather than ascorbate oxidase was added; the difference is due specifically to AA (reduced form only). Change in absorbance (0–1 min for AA and 0–4 min for FRAP) is converted to mmol/l by comparison with a standard of known AA concentration or FRAP value. Aqueous solutions of ferrous ions (iron(II) sulfate

(FeSO4.7H2O)) and ascorbic acid (L(1)-AA, extra pure crystals) both from Merck (Darmstadt, Germany) are used as calibrators. The FRAP assay has a limit of detection of 2mmol/l, and precision is excellent: within- and between-run CV are ,0:5 and 1:0 % respectively at between 500 and 2000 mmol/l antioxidant– reducing power, n.8 in each case. For AA, within- and between-run CV of FRASC are ,5 % at 25, 50, 100, and 440 mmol/l. All reagents and solutions were prepared in Milli-Q water, which was made from a Millipore ultrapure water system (Millipore Corp., Bedford, MA, USA).

FRASC reagents were as follows: 300 mmol/l acetate buffer, pH 3:6, prepared by dissolving 3:1 g sodium acetate trihydrate in distilled water, with 16 ml glacial acetic acid (BDH Laboratory Supplies, Poole, UK) added and made up to 1 litre with distilled water; 10 mM-2,4,6 tripyridyl-S-triazine (Fluka Chemicals, Buchs, Switzerland) solution in 40 mM-HCl (BDH Laboratory Supplies); 20 mM-FeCl3.6-H2O (BDH Laboratory Supplies) solution in distilled water. Working FRASC reagent was prepared as needed by mixing 10 ml acetate buffer with 1 ml 2,4,6tripyridyl-S-triazine solution and 1 ml FeCl3.6H2O solution. A 4 IU ascorbic oxidase/ml solution (Sigma Chemical Co., St, Louis, MO, USA) was prepared in distilled water, divided into portions and stored at 2708C until needed. For FRASC analysis, 100 ml of each freshly prepared food extract was mixed with 40 ml ascorbic oxidase solution; a matching (paired) 100 ml aliquot of each extract was mixed with 40 ml water; paired extracts were immediately loaded on the analyser (CobasFara centrifugal analyser; Roche Diagnostics Ltd, Basel, Switzerland). The 0–1 min change in A593 nm readings of the paired extracts (tested in parallel) were retrieved and used to calculate the AA concentration. The 0–4 min changes in A593 nm of the extracts treated with water only (no ascorbic oxidase) were retrieved and used to calculate the FRAP values. The FRAP value in mmol/l was calculated by simple comparison of 0–4 min change in absorbance at 593 nm of the test sample and that of a Fe21 calibrator, as follows: 0–4 min DA593 nm of test sample 0–4 min DA593 nm of standard ½Fe21Š standard ðmmol=lÞ: The AA value in mmol/l was calculated by comparison of 0–1 min change in absorbance at 593 nm of the extract and that of an AA calibrator, as follows: 0–1 min DA593 nm ¼ ð0–1 min DA593 nm sample 2 aoÞ 2 ð0–1 min DA593 nm sample 1 aoÞ; and AA concentration ðmmol=lÞ ¼ 0–1 min DA593 nm of test sample 0–1 min DA593 nm of standard ½AAŠ standard ðmmol=lÞ; where ao is ascorbic oxidase. AA was converted from mmol/l extract to mg/kg wet weight of fruit

or vegetable by simple calculation based on molecular mass of AA (176), the weight of fruit or vegetable extracted and the volume of fluid used for extraction (typically 5 g and 100 ml respectively). The % contribution of AA (which has a stoichiometric value of 2 in the FRAP assay, i.e. 1 mol AA reduces 2 mol Fe31 to Fe21) to the total antioxidant capacity (FRAP value) of each fruit or vegetable tested was calculated as follows: AA ðmmol=lÞ 2 FRAP value ðmmol=lÞ 100 %.

4.5.3. ANTI-INFLAMMATORY ACTIVITYOF SIDDHA FORMULATION RASA KARPOORA KULIGAI

The anti-inflammatory activities of ***RASA KARPOORA KULIGAI*** **at** a dose of 200 and 400mg/kg were evaluated using carrageenan-induced paw edema method. The inflammation was readily produced in the form of edema with the help of irritant such as carrageenan. Carrageenan is a sulphated polysaccharide obtained from sea weed (Rhodophyceae) and when injected cause the release of prostaglandins by the way it produces inflammation and edema.

REQUIREMENTS:

Animal	:	Albino rat (180-200 g)
Drugs and chemicals	:	Carrageenan (1%w/v), Diclofenac sodium (standard), Carboxy methyl cellulose (1%w/v), Digital plethysmo meter. U G O Basile (Italy)
Test compounds	:	siddha formulation *Rasa karpoora kuligai*

METHOD:

Anti-inflammatory activity was performed by the following procedure of Bhandri et al(1) The animals were divided into 4 groups each having six animals. A freshly prepared suspension of carrageenan (1% w/v , 0.1 ml) was injected to the planter region of left hind paw of each rat. One group was kept as control and the animals of the other groups were pretreated with the siddha formulations test Compounds dissolved with 2 ml sterile water given through orally 30 min before the carrageenan treatment. The paw volumes of the test compounds, standard and control groups were measured at 60,240,360 minutes of carrageenan treatment with the help of Digital plethysmometer (Ugo basile, Italy). Mean increase in paw volume was measured and the percentage of inhibition was calculated.

% Anti-inflammatory activity = (Vc-Vt / Vc) **x** ***100***

Where, ***Vt***-mean increase in paw volume in rats treated with test compounds,

Vc-mean increase in paw volume in control group of rats.

5. MICROBIOLOGICAL ANALYSIS

ANTI - MICROBIAL STUDIES

Aim

To study the Anti-microbial action of "Rasa karpoora kuligai'' done by **"Paper disc agar diffusion method"** – Kirby – bauyermethod.

Components of Muller Hinton agar medium

Beef extract	-	300gms/lit
Agar	-	17 gms/lit
Starch	-	1.5 gms/lit
Casein Hydrolysate	-	17.5 gms/lit
Distilled water	-	1000 ml
PH	-	7.6

Procedure:

The method of antibacterial activity study is UPS Diffusion Method. Antibiotic discs are prepared with known concentration of antibiotic are placed on agar plates that has been inoculated with the known pathogenic micro organism. The antibiotic diffuses through the agar producing an antibiotic concentration, gradient antimicrobial susceptibility is proportional to the diameter of the inhibitory zone around the disc. If the microorganism which grows up to the edge of the disc are resistant to the antimicrobial agent. The recommended medium in this method is Muller Hinton Agar, its PH should be between 7.2-7.6 and should be poured to uniform thickness of 4mm in the petri plate (25ml).

Methodology:

Muller Hinton Agar plates are prepared and Pseudomonas, Staphylococcus, Candida, Escherichia coli, Streptococcus is inoculated separately.

The prepared disc of *Rasa karpoora kuligai* are placed over the incubated plate using sterile forceps and incubated for 24 hours at 37 degree celcius. The plates after 24 hours incubation are observed for the zone of inhibition.

6. RESULTS AND DISCUSSION

Many studies have been carried out to bring the efficacy and potency of the drug ***Rasa karpoora kuligai***. The study includes literary collections, organoleptic character, physicochemical and instrumental analysis, pharmacological studies, and toxicological study. The drug ***Rasa karpoora kuligai*** has been selected for Anti-Cancer, Anti-oxidant, Anti-inflammatory activity in reference with the text ***"GUNAPADAM THATHU JEEVAM".***

- Literature collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the cancer.
- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in treating cancer.
- Pharmaceutical review describes about the kuligai and its properties.
- The pharmacological review explains about the methodology of Anti-cancer, Anti-oxidant, Anti-inflammatory activity and the drugs used.
- Modern and siddha aspect of the disease was also reviewed.

STANDARDISATION OF THE TEST DRUG

Standardisation of the drug is more essential to derive the efficacy and potency of the drug, which was analysed by the various methods. The results of physicochemical and biochemical analysis have been done and tabulated. Pharmacological activity and toxicological results of the drug were derived. The result reveals the effectiveness of the trial drug ***Rasa karpoora kuligai*** has been proved by the following scientific parameters.

Table -13.ORGANOLEPTIC CHARACTER

The following characters have been noted in ***Rasa karpoora kuligai***.

Table -5. Organoleptic characters

Colour in day light	Brown
Smell	Pleasant odour
Taste	Bitter, L.Pungent
Appearance	Round
Touch	Hard

Table – 14.Physicochemical properties

Table S.NO	**Parameter**	**Result**
	Loss on drying	4.4 %
	Ash content	7.3%
	Acid insoluble ash	2.4%
	Water sol.matter	34.7%
	Alcohol sol.matter	21.1%
	Microbial contamination Total bacterial count Total fungal count	 1.2×10^4col/g 2.1×10^2col/g
	Test for specific Pathogen E.coli Salamonella spp. S.aureus Pseudomonas aeruginosa	 Nil

INTERPRETATION:

Total Ash: Total ash value of plant material indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (ammonium, potassium, calcium, chloride, iron, etc.,) present in the drug is measured through the Total ash value and it is of 7.3 % for DDC.

Acid insoluble ash: The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is 2.4% for DDC.

Water soluble ash: Water-soluble ash is the part of the total ash content, which is soluble in water. It is 34.7% for DDC

Alcohol soluble ash: Alcohol-soluble ash is the part of the total ash content, which is soluble in alcohol. It is 21.1% for DDC

- These are indicating the approximate measure of chemical constituents of crude drug.
- The percentage of soluble matters present in the drug is determined by the values of water extractive and ethanol extractive.
- Based on the extractive value suitable solvent can be selected. It also gives the percentage of drug which will correlate with the metabolism reactions.
- Water-soluble extractive value plays an important role in evaluation of crude drugs
- The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value

Loss on drying:

- The total of volatile content and moisture present in the drug was established in loss on drying.
- Moisture content of the drug reveals the stability and its shelf-life.
- High moisture content can adversely affect the active ingredient of the drug.
- Thus low moisture content could get maximum stability and better shelf life.

THIN LAYER CHROMATOGRAPHY

Under UV 254 nm and 366 nm

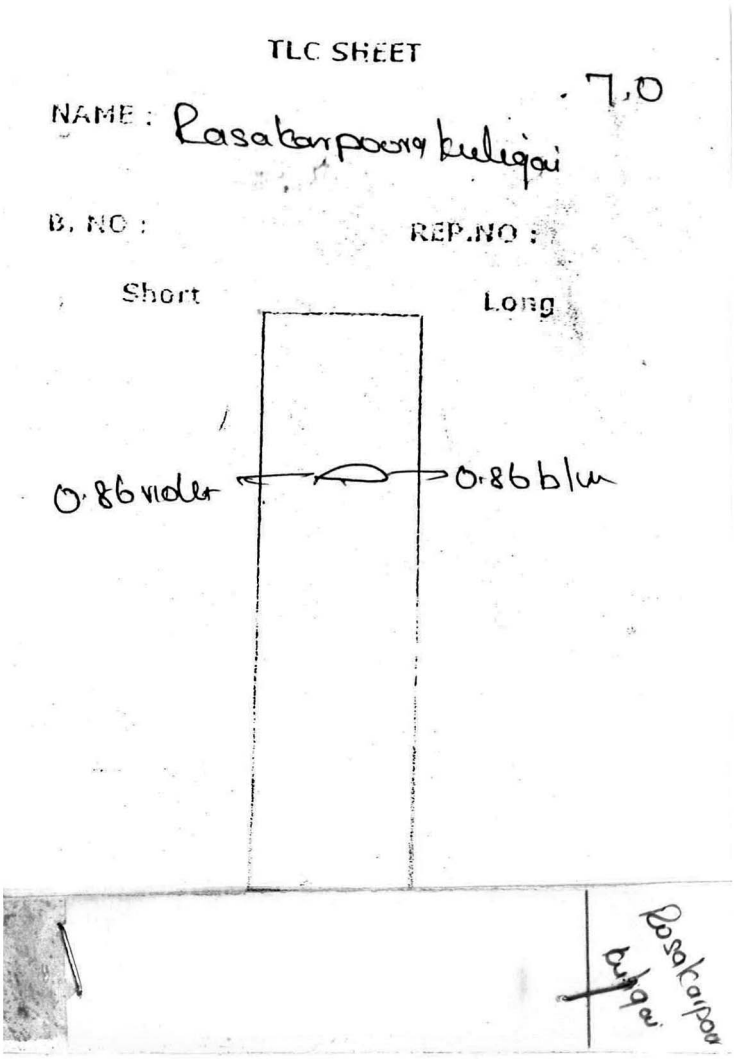


Fig. 2

INTERPRETATION:

Under UV 254 nm and 366 nm test related to alkaloids, it shows major spots short at Rf 0.86 (violet), 0.86 (blue). 2 major compounds are found.

BIO CHEMICAL ANALYSIS:

Table 7-Result of basic and acidic radical's studies:

Parameter	Result
Test for **Calcium**	Present
Test for **Sulphate**	Present
Test for **Chloride**	Present
Test for carbonate	Absent
Test for **Starch**	Present
Test for Iron [Ferric]	Absent
Test for **Iron [Ferrous]**	Present
Test for **Phosphate**	Present
Test for Albumin	Absent
Test for Tannic acid	Absent
Test for **Unsaturation**	Present
Test for the Reducing sugar	Absent
Test for Amino acid	Absent
Test for Zinc	Absent

From the basic and acidic radical studies of presents the following chemicals.

- Calcium
- sulphate
- Chloride
- Starch
- Ferrous iron
- phosphate
- Unsaturated compound

INTERPRETATION:

1. **Calcium**

Calcium is a major component of bones and teeth. It also is required for the clotting of blood to stop bleeding and for normal functioning of the nerves, muscles, and heart.Individuals who had a calcium intake of more than 700 mg per day had a 35 percent to 45 percent reduced risk of cancer of the distal (lower) part of the colon. In a study that included more than 61,000 Swedish women, colorectal cancer risk was approximately 28 percent lower among individuals who had the highest calcium intakes (approximately 800–1000 mg per day). High intakes of total calcium, dietary calcium, and supplemental calcium were associated with an approximately 20 percent lower risk of colorectal cancer

2. **Chloride :**

Chloride regulates the acid base balance of the body fluids, by maintaining the osmotic pressure of the body fluids. In severe diarrhoea vomiting, large amount of water and electrolytes are lost from body. The dehydraction has to be treated by administering water and these electrolytes.

3. **Ferrous Iron :**

Iron is easily soluble and readily absorbed from intestine and involved

4. **Starch:**

Starches functions much like dietary fibre. They provide nutrition for the beneficial bacteria in the colon, keeping them thriving and health. Dietary fibre in starch reduces effects of haemorrhoids, diverticulosis & controls blood pressure.

5. **Reducing sugar:**

Reducing sugars can react with other parts of the food like aminoacids to change colour and taste of the food.

Reducing sugars like Glucose is essential for brain function and physical energy

6.Phosphate.

Carcinoma of prostate (CaP), the most common malignancy in men, is also the second most common cause of cancer deaths in men.Phosphate reduce the risk for the development of prostatic diseases, both benign prostatic hyperplasia and Ca.

INSTRUMENTAL ANALYSIS

ICP-OES *(Inductively Coupled Plasma Optical Emission Spectroscopy):*

The drug *(Rasa karpoora kuligai)* sample was analysed by the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of ICP-OES is given on the Table.

Table – 15.ICP-OES of *Rasa karpoora kuligai*

S. no	Elements	Wavelength (nm)	Concentration
1.	Al	396.152	BDL
2.	As	188.979	BDL
3.	Ca	315.807	**11.160 mg/L**
4.	Cd	228.802	BDL
5.	Cu	327.393	BDL
6.	Fe	238.204	BDL
7.	Hg	253.652	BDL
8.	K	766.491	**13.801 mg/L**
9.	Mg	285.213	BDL
10.	Na	589.592	**14.310 mg/L**
11.	Ni	231.604	BDL
12.	Pb	220.353	BDL
13.	P	213.617	**86.321 mg/L**
14.	S	180.731	BDL
15	Zn	206.200	**01.058 mg/L**

BDL:Below Detectable Limit

1% = 10000ppm,

1ppm = 1/1000000 or 1ppm = 0.0001%

Table : 16 The toxic metals and the permissible limits

Heavy metals	WHO & FDA limits
Arsenic (As)	10ppm
Mercury (Hg)	1ppm
Lead (Pb)	10ppm
Cadmium (Cd)	0.3ppm

INTERPRETATION:

- Optical Emission spectrometry is based on the principle that atoms or ions in an excited state tend, to revert back to the ground state and in so doing emit characteristic wavelength and intensity of that light is proportional to the concentration of that particular element in the sample solution.
- This technique is used for quantitative and qualitative determination of the metals and mettaloids, in the biological preparation.
- The main ingredient of the drug is Mercuric II chloride, but the final product shows below detection limit of the Mercury. This results shows Below detection limit(BDL) of As(arsenic),Hg(Mercury), Cd (Cadmium), Pb(Lead), Ni(Nickel), Al(Aluminium), Cu (Copper).It is evident that the effectiveness of siddha medicine has been proved by the modern scientific way.
- This result indicates the presence of Calcium, Potassium, Sodium, Phosphorus, Zinc,

Calcium is a major component of bones and teeth. It also is required for the clotting of blood to stop bleeding and for normal functioning of the nerves, muscles, and heart.Individuals who had a calcium intake of more than 700 mg per day had a 35 percent to 45 percent reduced risk of cancer of the distal (lower) part of the colon. In a study that included more than 61,000 Swedish women, colorectal cancer risk was approximately 28 percent lower among individuals who had the highest calcium intakes (approximately 800–1000 mg per day). High intakes of total calcium, dietary calcium, and supplemental calcium were associated with an approximately 20 percent lower risk of colorectal cancer

Potassium is one of the major electrolytes that the body carefully controls for proper heart and neuromuscular activity. A high potassium blood level, or hyperkalemia, is seen in a variety of medical conditions, including cancer and cancer

treatment. Prompt recognition and treatment of hyperkalemia are important to avoid potentially life-threatening side effects.

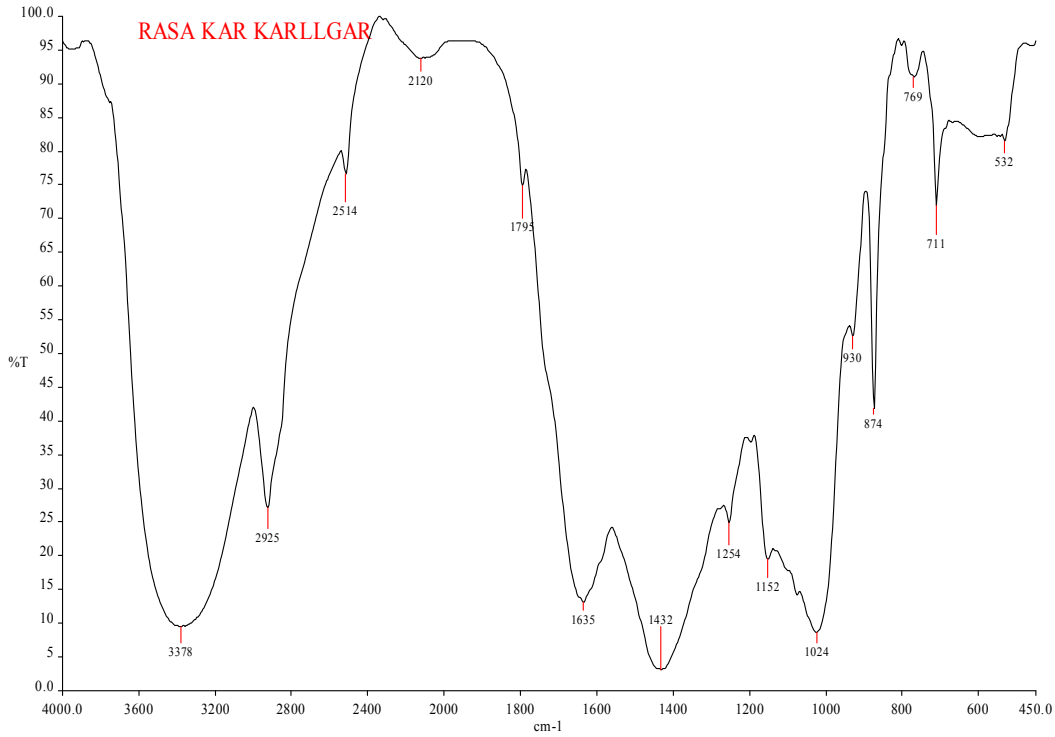
Sodium is one of the major electrolytes, carefully maintained within a narrow normal range and necessary for proper functioning of the body's systems. Low sodium levels, or hyponatremia, occur in a wide variety of medical disorders including cancer. Low sodium levels can occur in patients whose cancers produce ADH-like hormones, from other cancer complications and different treatment side effects. Cancers such as small cell lung carcinoma, pancreatic cancer, lymphoma and certain brain tumors can cause due to low sodium level.

Phosphate. Carcinoma of prostate (CaP), the most common malignancy in men, is also the second most common cause of cancer deaths in men.Phosphate reduce the risk for the development of prostatic diseases, both benign prostatic hyperplasia and Ca.

Zinc is essential for growth. It has been used in traditional medicine for everything from healing wounds to preventing blood clots.

- It can also help increase the blood flow.
- It has anti-oxidant properties that protect the cells from damage.
- It can produce healthy veins and arteries that enhance the blood circulation.

Fig : 3 FT IR SPECTROSCOPIC STUDIES OF RASA KARPOORAKULIGAI



Graph of FTIR analysis-FI

FTIR Spectrum- functional groups

Absorption peak	**Bond**	**Functional groups**
3378 (s,b)	O–H stretch,H–bonded	alcohols, phenols
2925 (m)	C–H stretch	alkanes
22120(w)	–C≡C– stretch	alkynes
1635(m)	N–H bend 1°	amines
1432 (m)	C–C stretch (in–ring)	aromatics
1254 (m)	C–H wag (–CH_2X)	alkyl halides
1152 (m)	C–H wag (–CH_2X)	alkyl halides
1024 (m)	C–N stretch	aliphatic amines
930 (m)	O–H bend	carboxylic acids
874 (s)	C–H “oop”	aromatics
769 (m)	C–Cl stretch	alkyl halides
711 (m)	C–H rock	alkanes
532 (m)	C–Br stretch	alkyl halides

INTERPRETATION

- FTIR instrumental analysis was done. The test drug was identified to have 13 peaks. They are the functional groups present in the trial drug *Rasa karpoora kuligai*.
- The above table shows the presence of alcohols,phenols,carboxylic acids,amines, aliphatic amines, aromatic,alkyl halides groups which represents the peak value.
- **PHENOLS:**

  Natural phenolic compounds play an important role in cancer prevention and treatment. Phenolic compounds from medicine and dietary plants include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and others. Various bioactivities of phenolic compounds are responsible for their chemopreventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways
- **AMINES**:

  Acts as a neuro transmitter.Involved in protein synthesis.Amines play an important role in reducing abdominal pain, bloating.
- **ALKANES**:

  They protect against bacteria and fungal infections.
- **ALCOHOLS**:

  Has anti microbial action.Acts as a antiseptic agent.
- **ALDEHYDES**:

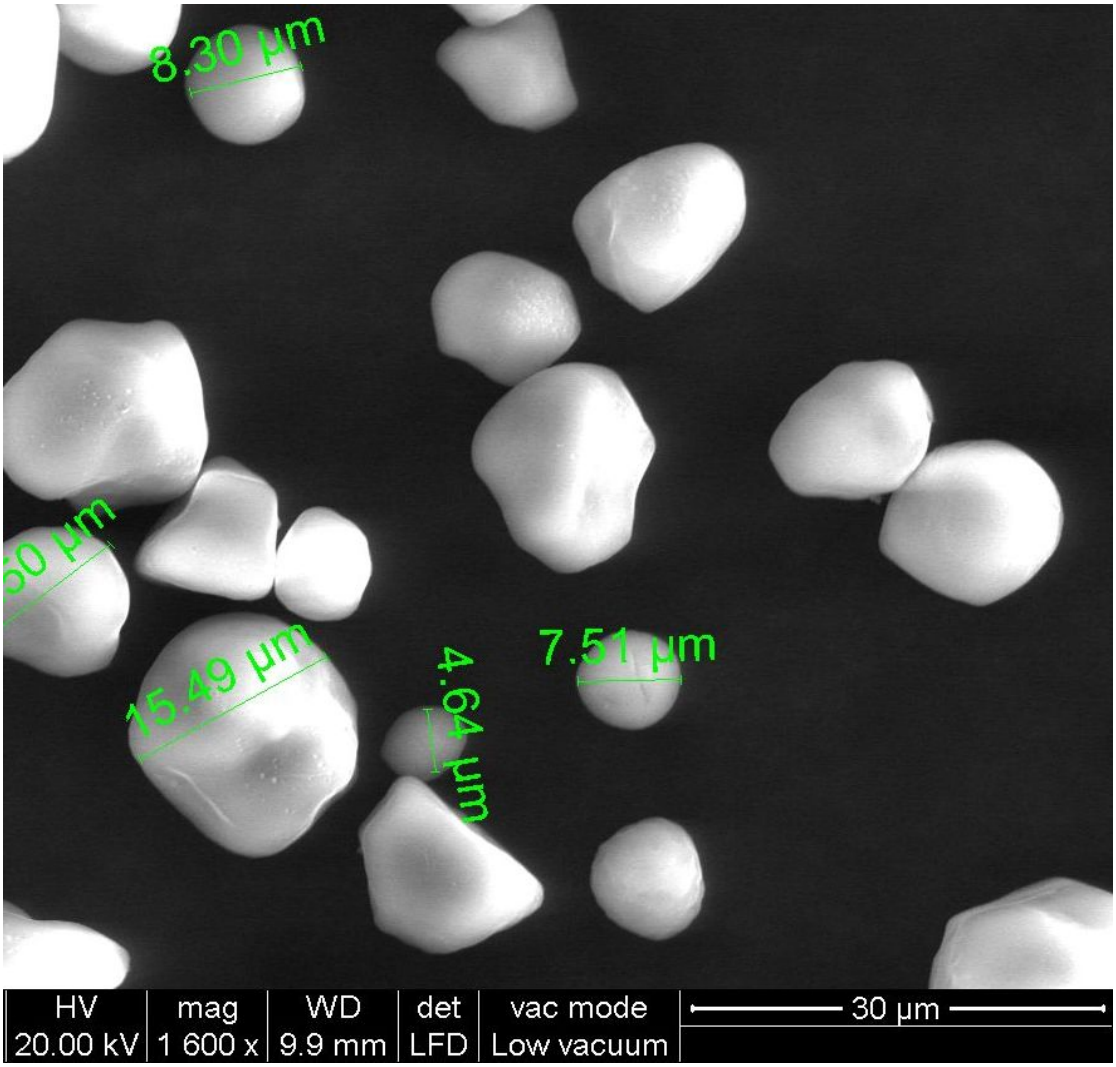
  Aldehydes normally have Anti-microbial activities.They are readily absorbed by the Gastro intestinal tract.
- **AROMATICS**:

  Aromatics are good pain relievers.Has Anti-pyretic,Anti-inflammatory,Auto-immune activities.

SCANNING ELECTRON MICROSCOPE *(SEM)*

The above SEM studies of microscopic resolution of 1.00kx and examining surface area of 800x800µm^2, showed objects of sizes ranging from 238nm to 506nm. The surface of the sample grains is uniformly arranged in agglomerates. They are micro particles ranging from 238nm, 506nm, 308nm, 341nm, 371nm.

FIG-4.SEM-PICTURE



SEM PICTURE 24,000 MAGNIFICATION.

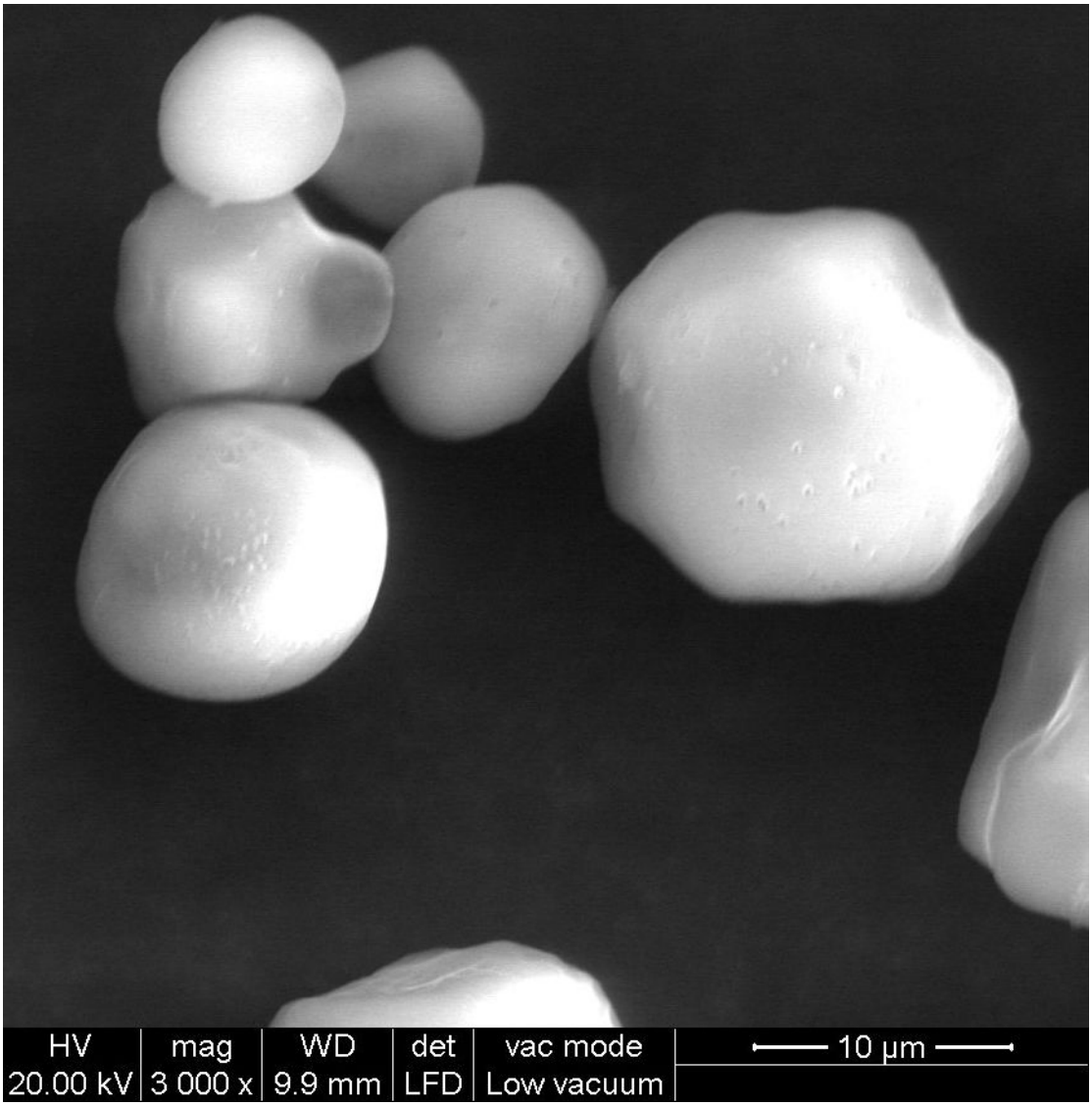


Fig 4 : SEM PICTURE 40,000 MAGNIFICATION.

INTERPRETATION

Scanning Electron Microscopy (SEM) is the best method to determine the size distribution of nanoparticles present in the sample. It has been used for this purpose of computer-assisted counting of nanoparticle images. SEM analysis of the ***Rasakarpoorakuligai*** shows that the uniform distribution of particles presents in the entire field. Most of the particles present in the sample is nano size and near nano size, average particle size is 4.64µm - 7.51µm.So, very minimal quantity of the medicine is enough to treat the disease.

Siddhars were the great scientist in ancient times. They used nano technology for the preparation of Medicines. These Nano particles have beneficial properties that can be used to improve drug delivery system. Target cells take up these nano particles quickly because of their smaller size, lesser particles enhance the bio absorption and bio availability resulting better efficacy of the drug. Larger particles could not enter in to the target cell because of their size, resulting they cleared from the body. If a drug is cleared too quickly from the body, this could force a patient to use high dose, which leads to contraindication of the drugs. The Lesser particles from drug delivery system lower the volume of distribution and reduce the effect on non target tissue. In Siddha system of medicine adjuvant and detoxification (Purification) is also important factors for drug transport.

EVALUATION OF ACUTE TOXICITY OF *RASA KARPOORA KULIGAI*

Effect of Acute Toxicity (14 Days) of *RASA KARPOORA KULIGAI*

Table: Shows Physical and behavioral examinations.

Table 18 Physical and behavioral examinations.

Group no.	Dose(mg/kg	Observation sign	No. of animal affected.
Group-I	5mg/kg	Normal	0 of 3
Group- II	50mg/kg	Normal	0 of 3
Group-III	300mg/kg	Normal	0 of 3
Group-IV	1000mg/kg	Normal	0 of 3
Group-V	2000mg/kg	Normal	0 of 3

Table-19 Home cage activity

Functional and Behavioural observation	Observation	5mg/kg Group (G-I)	50mg/kg (G-II)	300mg/kg (G-III)	1000mg/kg (G-IV)	2000mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Body position	Normal	3	3	3	3	3
Respiration	Normal	3	3	3	3	3
Clonic involuntary Movement	Normal	3	3	3	3	3
Tonic involuntary Movement	Normal	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3
Approach response	Normal	3	3	3	3	3
Touch response	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Pinna reflex	Normal	3	3	3	3	3
Tail pinch response	Normal	3	3	3	3	3

Table-20 Hand held observation

Functional and Behavioral observation	Observation	Control	5 mg/ kg (G-I)	50 mg/kg (G-II)	300 mg/kg (G-III)	1000 mg/kg (G-IV)	1750 mg/kg (G-V)
		Female n=3	Female n=3	Female n=3	Female n=3	Female n=3	Female n=3
Reactivity	Normal	3	3	3	3	3	3
Handling	Normal	3	3	3	3	3	3
Palpebral closure	Normal	3	3	3	3	3	3
Lacrimation	Normal	3	3	3	3	3	3
Salivation	Normal	3	3	3	3	3	3
Piloerection	Normal	3	3	3	3	3	3
Pupillary reflex	Normal	3	3	3	3	3	3
Abdominal tone	Normal	3	3	3	3	3	3
Limb tone	Normal	3	3	3	3	3	3

Table-21 DOSE CONCENTRATION- 2000mg/kg

S.N	Response	Head		Body		Tail	
		Before	After	Before	After	Before	After
1	Alertness	N	-	N	-	N	-
2	Grooming	A	-	A	-	A	-
3	Touch response	P	-	P	-	P	-
4	Torch response	P	-	P	-	P	-
5	Pain response	A	-	A	-	A	-
6	Tremors	A	Present	A	Present	A	Present
7	Convulsion	A	Present	A	Present	A	Present
8	Righting reflux	A	Present	A	Present	A	Present
9	Gripping strength	P	-	P	-	P	-

10	Pinna reflux	P	-	P	-	P	-
11	Corneal reflux	P	-	P	-	P	-
12	Writhing	A	-	A	-	A	-
13	Pupils	N	-	N	-	N	-
14	Urination	N	-	N	-	N	-
15	Salivation	A	-	A	-	A	-
16	Skin colour	N	-	N	-	N	-
17	Lacrimation	A	-	A	-	A	-
18	Hyper activity	A	Present	A	present	A	Present

Table-22 Mortality

Group no	**Dose no(mg/kg)**	**Mortality**
Group-I	**5(mg/kg)**	**0 of 3**
Group-II	**50(mg/kg)**	**0 of 3**
Group-III	**300(mg/kg)**	**0 of 3**
Group-IV	**1000(mg/kg)**	**0 of 3**
Group-V	**1750(mg/kg)**	**0 of 3**
Group-VI	**2000(mg/kg)**	**3 of 3**

RESULT:

From acute toxicity study it was observed that the administration of **RASA KARPOORA KULIGAI** at a dose of 2000mg/kg, to a rats. Immediately after the administration the **RASA KARPOORA KULIGAI** at a dose of 2000mg/kg to develop hyperactivity, righting reflux, tremors and convulsion and death the RASA KARPOORA KULIGAI at a dose of 2000mg/kg to produce drug-related toxicity and mortality . From acute toxicity study it was observed that the administration of **RASA KARPOORA KULIGAI** at a dose of 1750 mg/kg to the rats do not produce drug-related toxicity and mortality. **So No-Observed-Adverse-Effect- Level (NOAEL) of RASA KARPOORA KULIGAI is 1750 mg/kg**.

INTERPRETATION:

RASA KARPOORA KULIGAI was administered single time at the dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 1750 mg/kg to rats and observed for consecutive 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of **RASA KARPOORA KULIGAI** at the doses of 5mg/kg, 50mg/kg, 300mg/kg, 1000mg/kg and 1750 mg/kg to rats. At the 14th day, all animals were observed for functional and behavioral examination. In functional and behavioral examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloercetion, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioral examination was normal in all treated groups. Food consumption of all treated animals was found normal as compared to normal group.

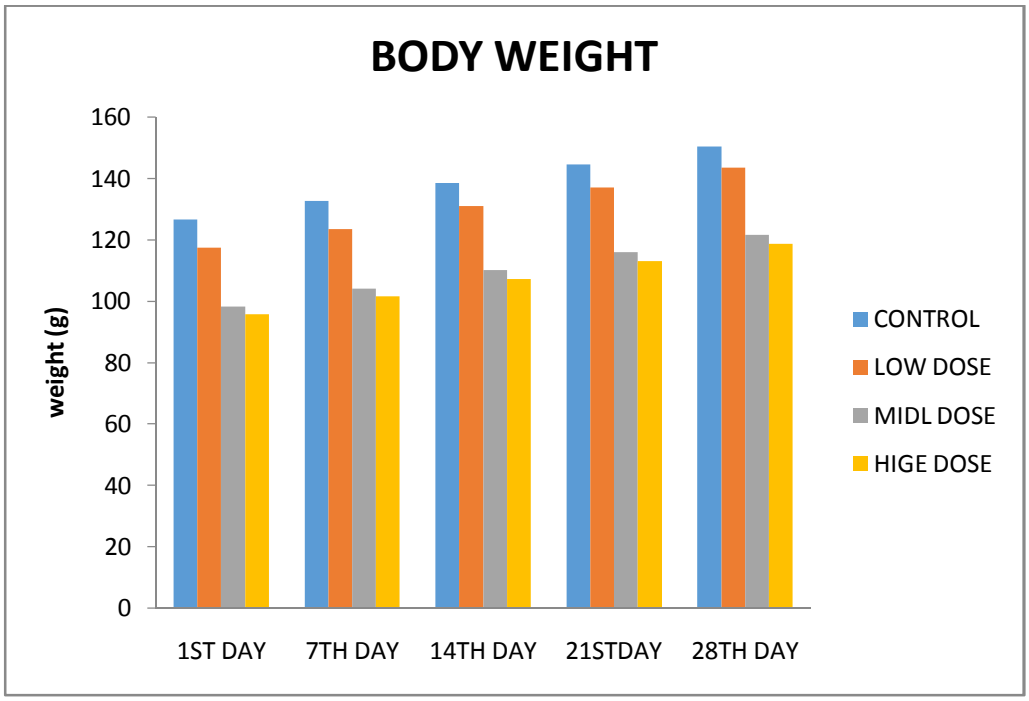
SUB-ACUTE TOXICITY RESULTS

Table : 23 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF RASA KARPOORA KULIGAION BODY WEIGHT IN GRAM

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGE DOSE 600mg/kg
0,DAY	126.667±1.42984	117.5±0.718795	98.3333±0.954521	95.8333±1.7966
7th DAY	132.667±1.42984	123.5±0.921955	104±1.06458	101.667±1.72562
14th DAY	138.5±1.40831	131±1.41421	110.167±1.27584	107.333±1.7062
21st DAY	144.5±1.40831	137±1.52753	116±1.18322	113±1.59165
28th DAY	150.333±1.52023	143.5±1.82117	121.667±1.22927	118.667±1.68655

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

Fig : 5



EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF RASA

Table : 24 KARPOORA KULIGAI

ON ORGAN WEIGHT (PHYSICAL PARAMETER) IN GRAM

GROUP		CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGE DOSE 600mg/kg
BRAIN		1.256±0.2052	1.232±0.1547	1.499±0.2023	1.159±0.2025
HEART		0.8727±0.04385	0.8323±0.06207	0.738±0.03329	0.6877±0.02028
LIVER		6.019±0.359	8.448±0.652	6.706±0.07517	5.779±0.338
LUNGS		1.447±0.1242	0.9353±0.03152	1.671±0.1854	1.278±0.2453
KIDNEY	L	0.7613±0.03038	0.8533±0.06263	0.8533±0.05191	0.7467±0.02533
	R	0.7453±0.03374	0.8287±0.06534	0.721±0.01721	0.7333±0.02809
TESTIS		3.095±0.1521	2.517±0.1654	3.295±0.08581	3.11±0.1176
UTERUS		0.736±0.02501	0.9607±0.05447	0.985±0.03436	1.214±0.306

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groupswith control group.

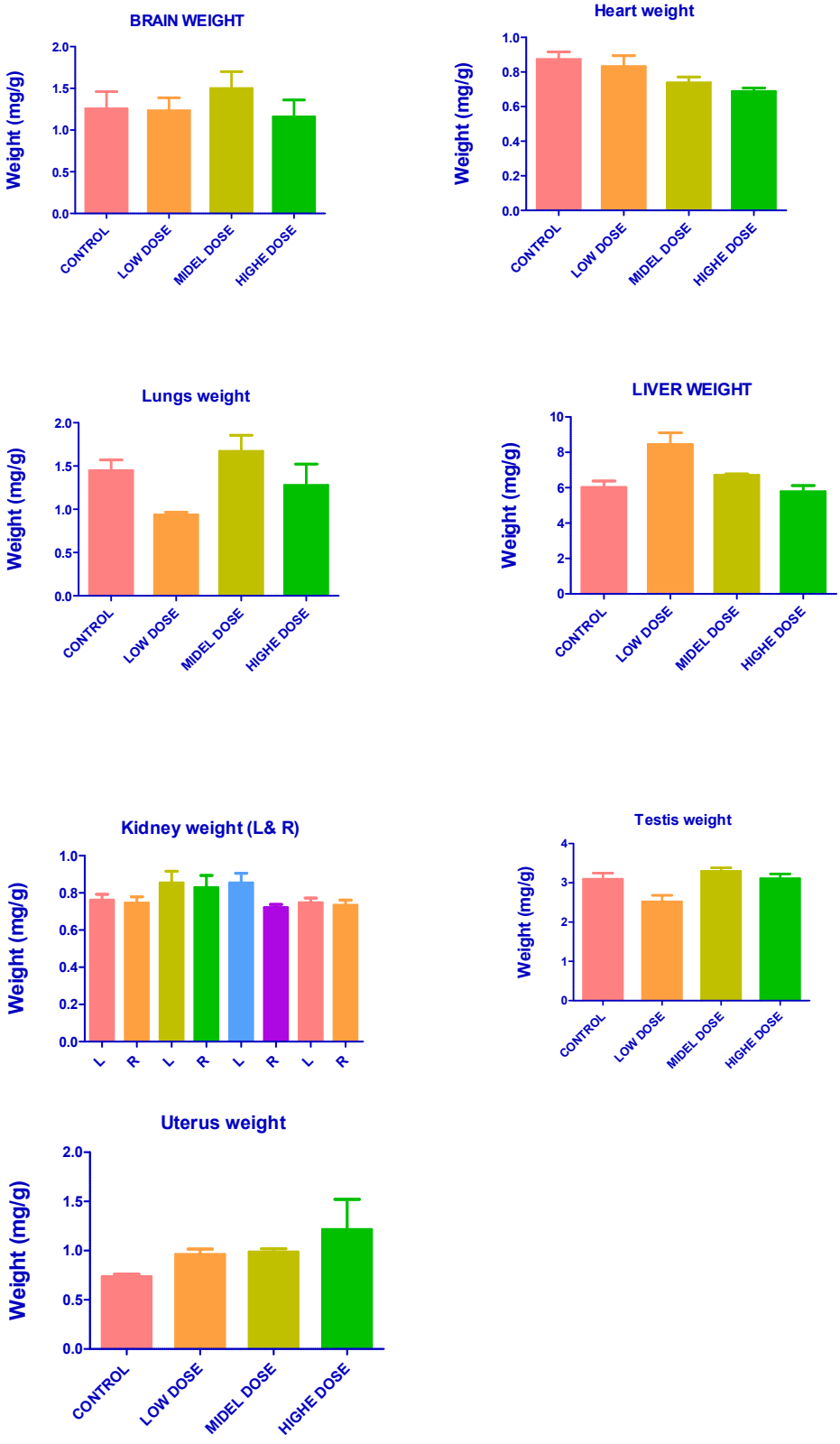

BRAIN WEIGHT
Weight (mg/g)
2.0
1.5
1.0
0.5
0.0
CONTROL
LOW DOSE
MIDEL DOSE
HIGHE DOSE
Heart weight
Weight (mg/g)
1.0
0.8
0.6
0.4
0.2
0.0
CONTROL
LOW DOSE
MIDEL DOSE
HIGHE DOSE
Lungs weight
Weight (mg/g)
2.0
1.5
1.0
0.5
0.0
CONTROL
LOW DOSE
MIDEL DOSE
HIGHE DOSE
LIVER WEIGHT
Weight (mg/g)
10
8
6
4
2
0
CONTROL
LOW DOSE
MIDEL DOSE
HIGHE DOSE
Kidney weight (L& R)
Weight (mg/g)
1.0
0.8
0.6
0.4
0.2
0.0
L
R
L
R
L
R
L
R
Testis weight
Weight (mg/g)
4
3
2
1
0
CONTROL
LOW DOSE
MIDEL DOSE
HIGHE DOSE
Uterus weight
Weight (mg/g)
2.0
1.5
1.0
0.5
0.0
CONTROL
LOW DOSE
MIDEL DOSE
HIGHE DOSE

Fig : 6

Tabel : 25 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF RASA KARPOORA KULIGAI ON HAEMATOLOGICAL PARAMETERS

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDDLE DOSE 400mg/kg	RKK. HIGH DOSE 600mg/kg
RBC (X10^6/µL)	4.573±0.1139	5.39±0.3035	4.853±0.6894	5.8±0.3617
WBC(X10^3/µL)	12.5±1.531	11.47±0.5783	13±1.007	11.43±0.3756
HB (g/dl)	10.5±0.5859	13.2±0.8963	11.83±1.683	14.03±0.809
PCV %	32.2±1.833	40.6±2.689	36.17±4.725	43.1±2.427
POLYMORPHS (%)	7.333±0.6667	10±1	8±3.055	7±1.155
LYMPHOCYTES (%)	85.33±2.028	81.33±1.856	79.33±3.528	85.67±2.963
MONOCYTES (%)	5±0.5774	3±0.5774	3.333±0.6667	3.333±0.3333
EOSINOPHILS (%)	3.333±0.3333	4.333±0.3333	4.667±0.8819	4.333±0.3333
MCH (Pg)	23.6±0.611	24.93±0.4702	24.83±0.6566	25.77±0.3283

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groupswith control group.

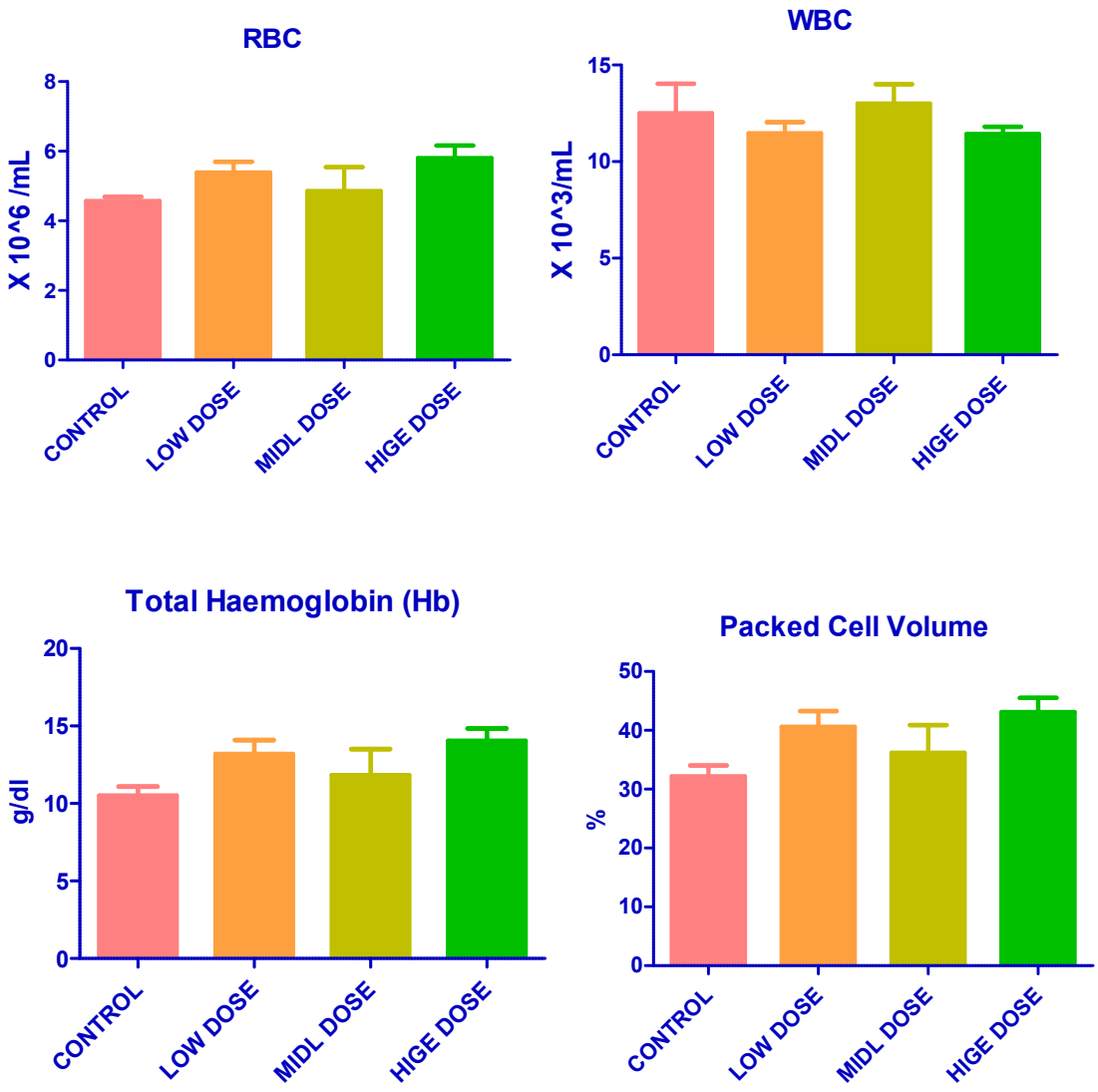

RBC
X 10^6 /mL
8
6
4
2
0
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
WBC
X 10^3/mL
15
10
5
0
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
Total Haemoglobin (Hb)
g/dl
20
15
10
5
0
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
Packed Cell Volume
%
50
40
30
20
10
0
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE

Fig : 7

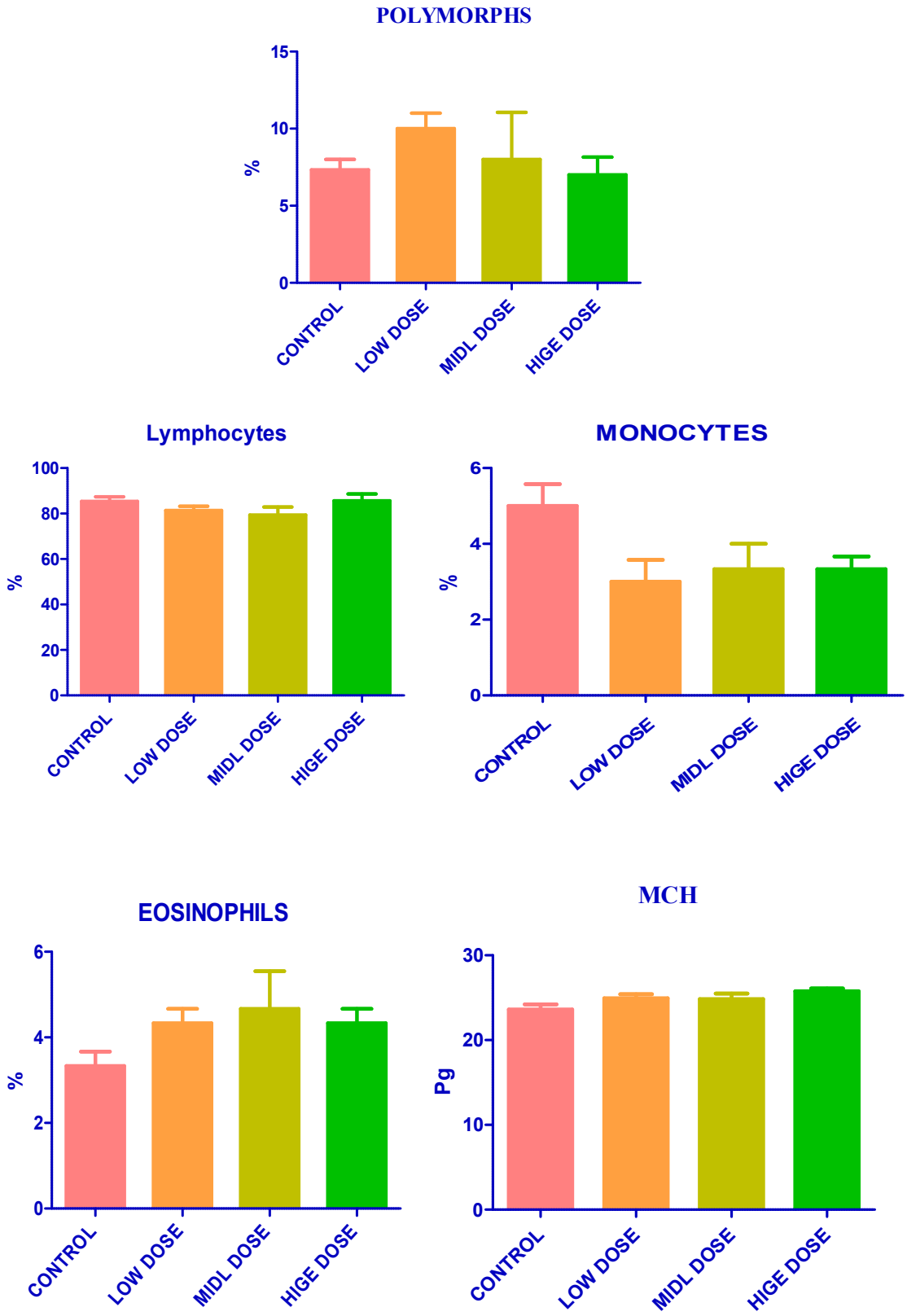

POLYMORPHS
15
10
5
0
%
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
Lymphocytes
100
80
60
40
20
0
%
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
MONOCYTES
6
4
2
0
%
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
EOSINOPHILS
6
4
2
0
%
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE
MCH
30
20
10
0
Pg
CONTROL
LOW DOSE
MIDL DOSE
HIGE DOSE

Fig : 7

Table : 26 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF RASA KARPOORA KULIGAI ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGH DOSE 600mg/kg
SGOT(units/min/liter/ mg protein)	97.2±6.835	116.1±11.08	98.37±10.16	91.5±3.523
SGPT(units/min/liter/ mg protein)	44.77±8.151	90.73±17.71	45.3±5.852	48.6±6.012
ALP (units/min/liter/mg protein)	210.1±69.74	175.9±11.87	169.9±27.35	186.6±24.95

EFFECT OF SUB- ACUTE DOSE(28 DAYS)OF RASA KARPOORA KULIGAI ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGH DOSE 600mg/kg
TOTAL BILIRUBIN (mg/dl)	1.303±0.2452	1.453±0.2822	0.8193±0.3371	0.99±0.194

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, calculated by comparing treated groupswith control group.

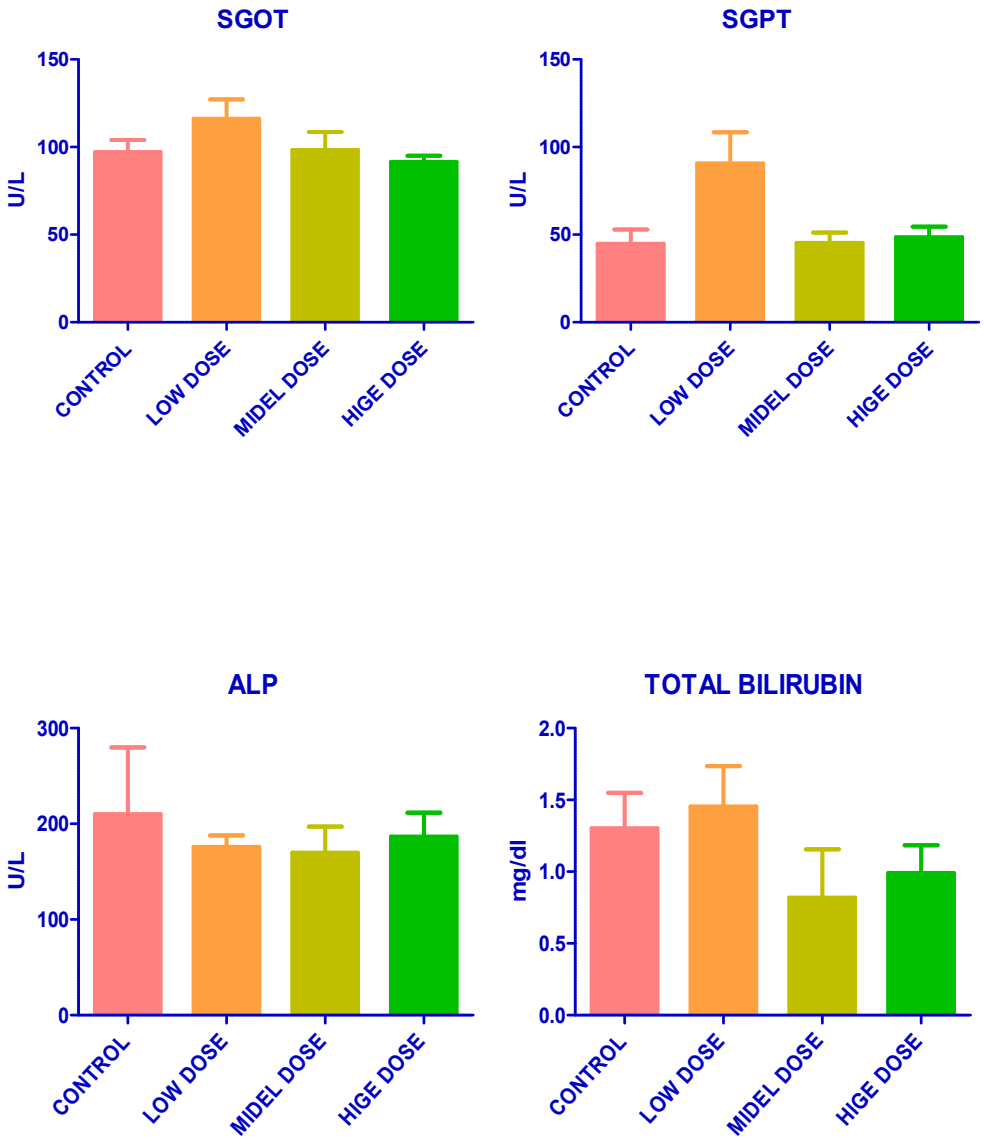

SGOT
U/L
150
100
50
0
CONTROL
LOW DOSE
MIDEL DOSE
HIGE DOSE
SGPT
U/L
150
100
50
0
CONTROL
LOW DOSE
MIDEL DOSE
HIGE DOSE
ALP
U/L
300
200
100
0
CONTROL
LOW DOSE
MIDEL DOSE
HIGE DOSE
TOTAL BILIRUBIN
mg/dl
2.0
1.5
1.0
0.5
0.0
CONTROL
LOW DOSE
MIDEL DOSE
HIGE DOSE

Fig : 8

Table : 26 EFFECT OF SUB- ACUTE DOSE (28 DAYS)OF *RASA KARPOORA KULIGAI* ON BIOCHEMICAL PARAMETERS

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGH DOSE 600mg/kg
CREATININE (mg/dl)	0.6133±0.03844	0.58±0.08327	0.4767±0.0441	0.5867±0.02028
URIC ACID (mg/dl)	1.747±0.2761	1.74±0.155	2.06±0.6612	1.983±0.2924

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

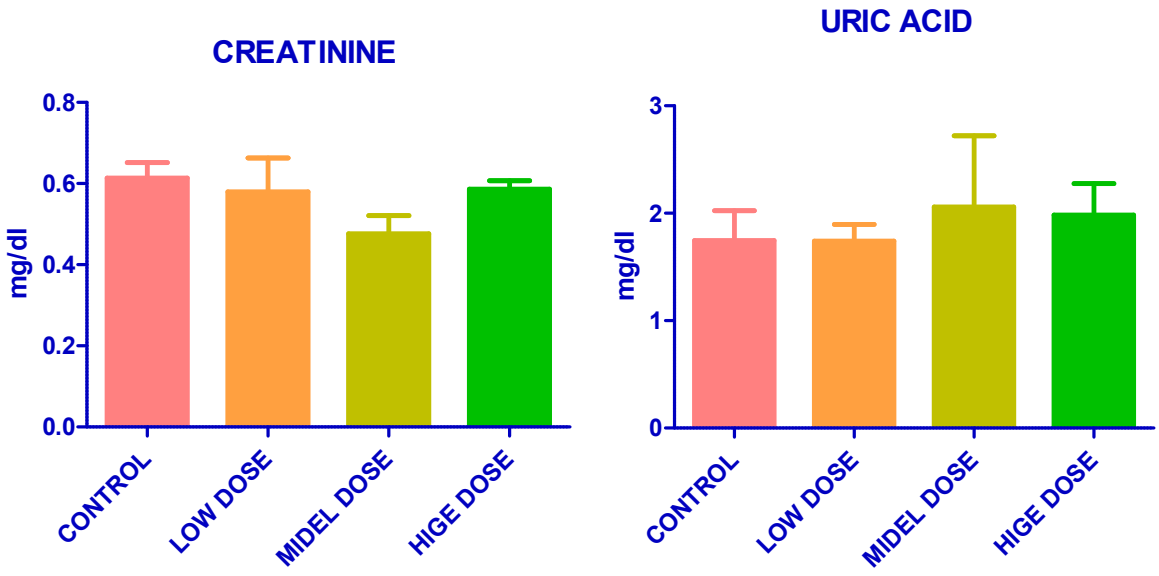


Fig : 7

Table : 27 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF RASA KARPOORA KULIGAI ON FOOD INTAKE IN GRAM

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGE DOSE 600mg/kg
1st DAY	42±4.53137	38±4.64758	54.6667±6.23253	40.6667±4.20053
7th DAY	35.1667±4.13454	39±6.90411	44.5±3.30404	60.6667±7.82588
14th DAY	42±4.14729	42.6667±5.92546	47±7.49667	47.3333±8.40106
21st DAY	52±5.83095	41.1667±6.5341	42.6667±9.04311	34.6667±5.71936
28th DAY	50.5±4.62421	47.3333±6.27517	37.3333±5.3831	42.1667±6.42089

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group

Table : 28 Effect of Sub- Acute Dose (28 Days) Of RASA KARPOORA KULIGAI On Water Intake in ml

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGE DOSE 600mg/kg
1st DAY	98.3333±13.5195	89.1667±14.3421	102.5±21.6699	67.5±7.6103
7th DAY	82.5±11.7438	100.833±12.6765	76.6667±9.80363	81.6667±14.4145
14th DAY	58.3333±8.72417	90.8333±14.2838	80±13.9642	89.1667±8.88976
21st DAY	91.6667±12.4944	80±8.46562	65.8333±9.43545	89.1667±8.79552
28th DAY	82.5±11.3835	88.3333±11.4504	75±8.85061	65±7.52773

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's(n=6); $^{ns}p>0.05$, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group

Fig : 9 EFFECT OF SUB- ACUTE DOSE (28 DAYS) OF RASA KARPOORA KULIGAI
ON FOOD INTAKE IN GRAM.

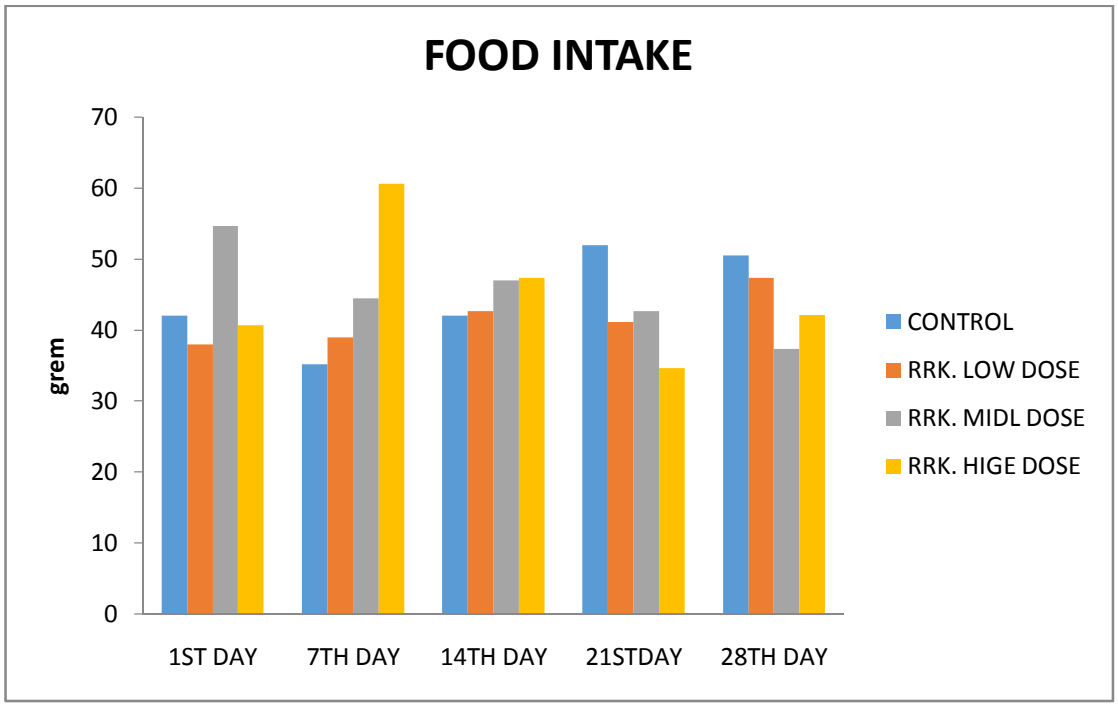


Fig : 9 Effect of Sub- Acute Dose (28 Days) Of RASA KARPOORA KULIGAI On Water Intake in ml

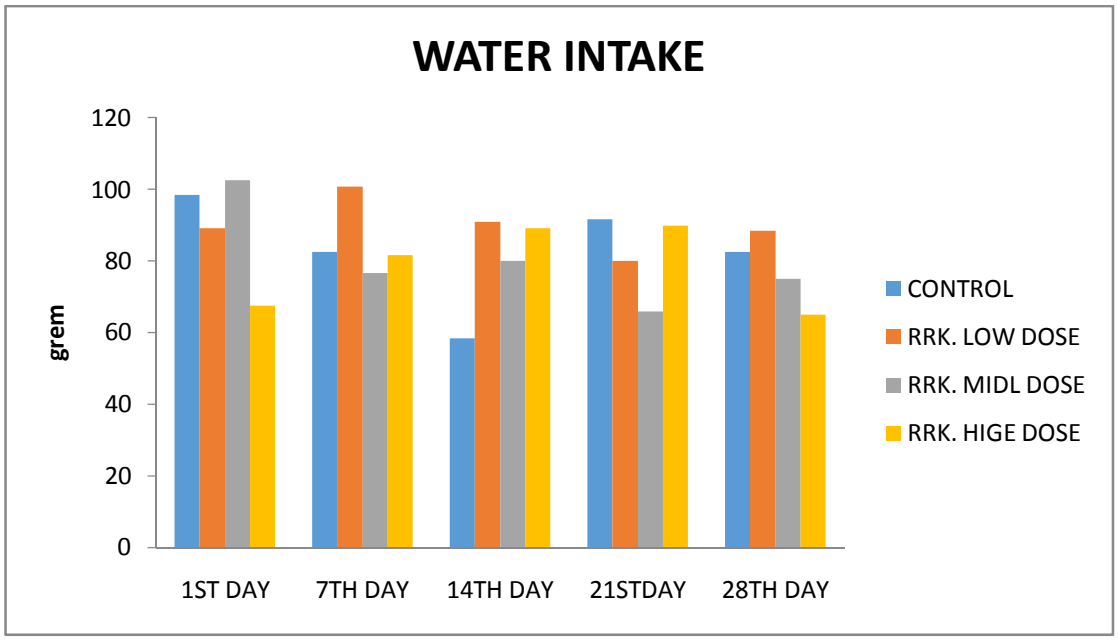


Table : 29 EFFECT OF SUB ACUTE DOSES (28 DAY) OF RASA KARPOORA KULIGAI ON ELECTROLYTES:-

GROUP	CONTROL	RKK. LOW DOSE 200mg/kg	RKK.MIDLE DOSE 400mg/kg	RKK. HIGE DOSE 600mg/kg
Sodium (mg/dl)	4.5±0.6455	4.25±0.6292	6±0.7071	6.75±0.75
Calcium (mg/dl)	1.575±0.137689	3.15±0.170783***	4.2±0.163299***	6.175±0.19311***
Phosphorus (U/L)	0.273±0.022517	0.3005±0.019615^{ns}	0.35625±0.030491^{ns}	5.033±0.32452*

Values arae expressed as mean ± SEM Statisticalsignificance (p) calculated by one way ANOVA followed by dunnett's (n=6); NS- non significant, *p<0.05, **p<0.01, ***p<0.001, calculated by comparing treated groupswith control group.

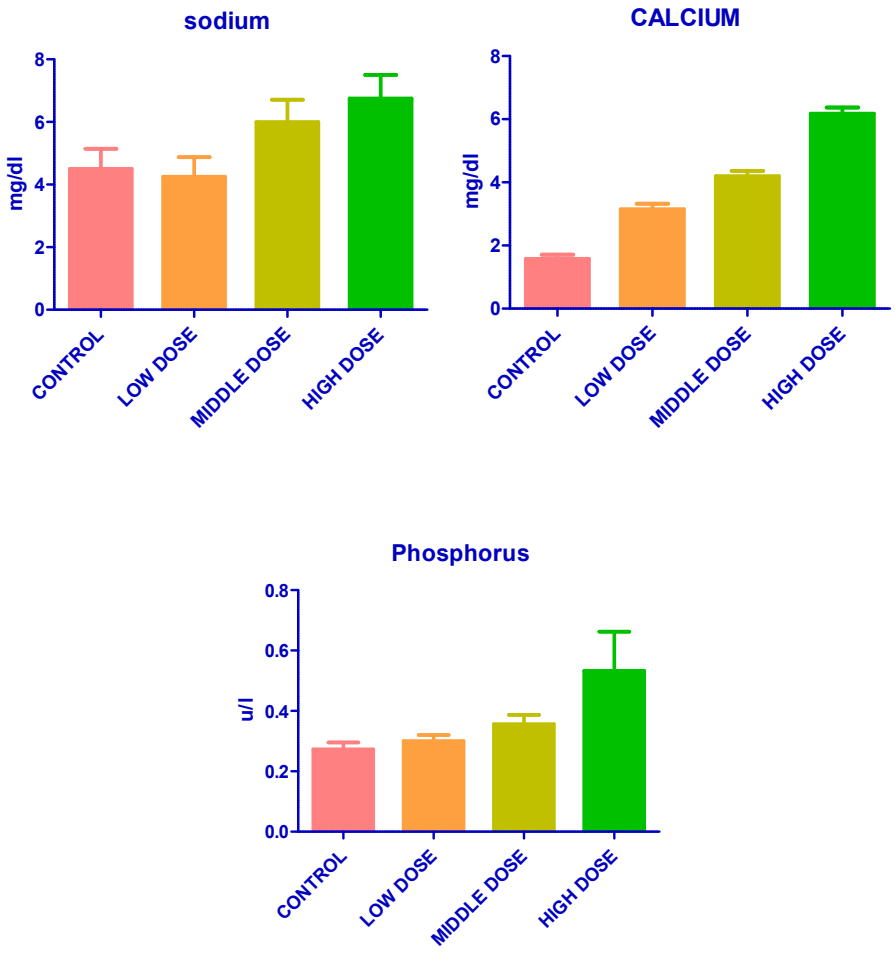


Fig : 11

RESULTS:

CLINICAL SIGNS:

All animals in this study were free of toxic clinical signs throughout the dosing period of 28 days.

Mortality:

All animals in control and in all the treated dose groups survived throughout the dosing period of 28 days.

Body weight:

Results of body weight determination of animals Table-1 from control and different dose groups exhibited comparable body weight gain throughout the dosing period of 28 days.

Food consumption:

During dosing and the post-dosing recovery period, the quantity of food consumed by animals from different dose groups was found to be comparable with that by control animals.

Organ Weight:

Group Mean Relative Organ Weights (%of body weight) are recorded in Table No.4 Comparison of organ weights of treated animals with respective control animals on day 29 was found to be comparable similarly.

Hematological investigations:

The results of hematological investigations (Table 4) conducted on day 29 revealed following significant changes in the values of different parameters investigated when compared with those of respective controls; however, the increase or decrease in the values obtained was within normal biological and laboratory limits or the effect was not dose dependent.

Biochemical Investigations:

Results of Biochemical investigations conducted on days 29 and recorded in Table 2 revealed the following significant changes in the values of hepatic serum enzymes studied. When compared with those of respective control. However, the increase or decrease in the values obtained was within normal biological and laboratory limits.

Histopathology:

In histopathological examination, revealed normal architecture in comparison with control and treated animal.

INTERPRETATION

1) All the animals from control and all the treated dose groups up to 600 mg/kg survived throughout the dosing period of 28 days.

2) No signs of toxicity were observed in animals from different dose groups during the dosing period of 28 days.

3) Animals from all the treated dose groups exhibited comparable body weight gain with that of controls throughout the dosing period of 28 days.

4) Food consumption of control and treated animals was found to be comparable throughout the dosing period of 28 days

5) Haematological analysis conducted at the end of the dosing period on day 29, revealed no abnormalities attributable to the treatment.

6) Biochemical analysis conducted at the end of the dosing period on day 29 no abnormalities attributable to the treatment.

7) Organ weight data of animals sacrificed at the end of the dosing period was found to be comparable with that of respective controls.

8) Histopathological examination revealed normal architecture in comparison with control and treated animal.

Table : 30 PHARMACOLOGICAL STUDY RESULT

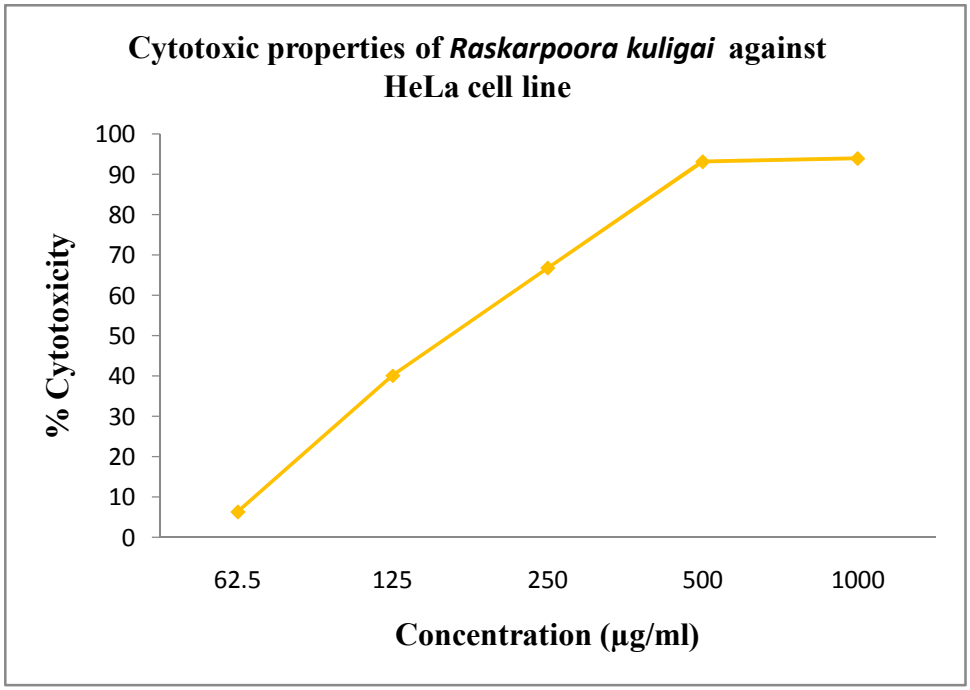
CYTOTOXIC PROPERTIES OF *RASA KARPOORA KULIGAI* AGAINST HELA CELL LINE.

Results:

Table -------: Cytotoxic properties of *Raskarpoorakuligai* against HeLa cell line.

Sl. No	Name of Test sample	Test Concn. (µg/ml)	% Cytotoxicity	CTC_{50} (µg/ml)
1	RKK	62.5 125 250 500 1000	6.32±1.3 40.12±0.2 66.84±1.0 93.22±0.1 94.02±0.2	164.77±1.6

FIG: 12 Graphical representation of cytotoxic effect of *Raskarpoorakuligai* on HeLa cell line



INTERPRETATION

Apoptosis is considered a vital component of various processes including normal cell turn over, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Inappropriate apoptosis (either too little or too much) is a factor in many human conditions including neurodegenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. The ability to modulate the life or death of a cell is recognized for its immense therapeutic potential.

Cytotoxicity activity:

The cytotoxicity study was carried out for Herbomineral drug ***Raskarpoorakuligai***. These extract was screened for its cytotoxicity against HeLa cell lines at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. Results are tabulated in Table --- and graphically represented in Fig. ---. The percentage growth inhibition was found to be increasing with different concentration of test compound.***Rasakarpoorakuligai*** effect on HeLa cell line at the concentration increased from 62.5 to 1000µg/ml, percentage of inhibition increased from 6.32 % to 94.02% and the IC50 value on HeLa cell line was 164.77µg/ml. Highest percentage of inhibition(94.02%)obtained at the concentration of 1000µg/ml. The results suggested that the ***Rasakarpoorakuligai*** inhibited the proliferation of human cervical cancer HeLa cells. Furtherstudies are needed to explore the intracellular mechanism.

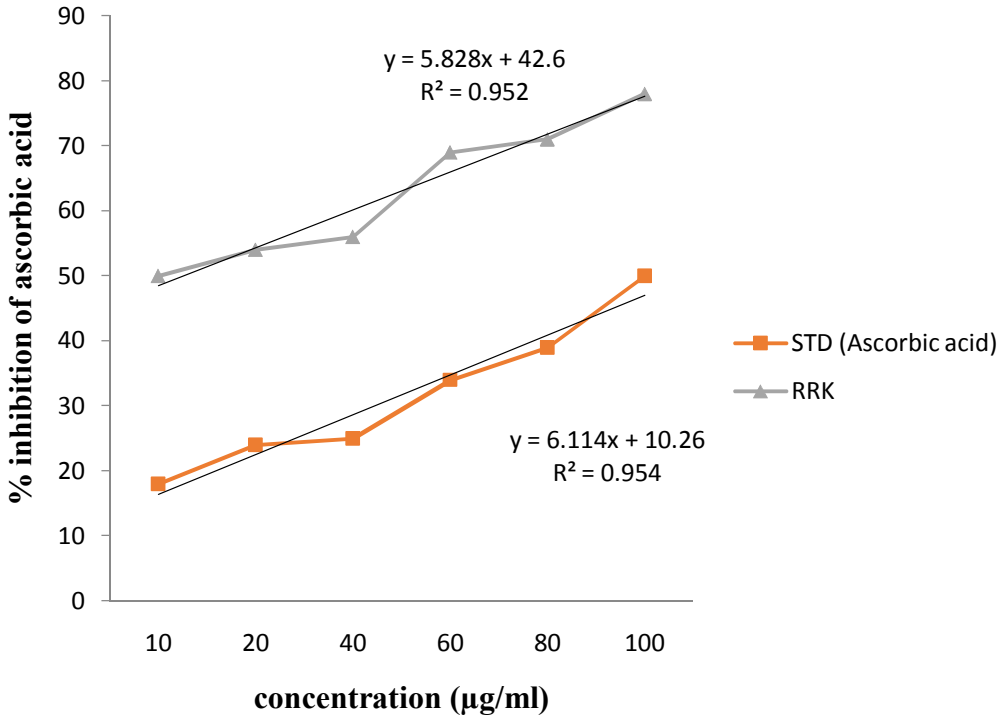
RESULTS

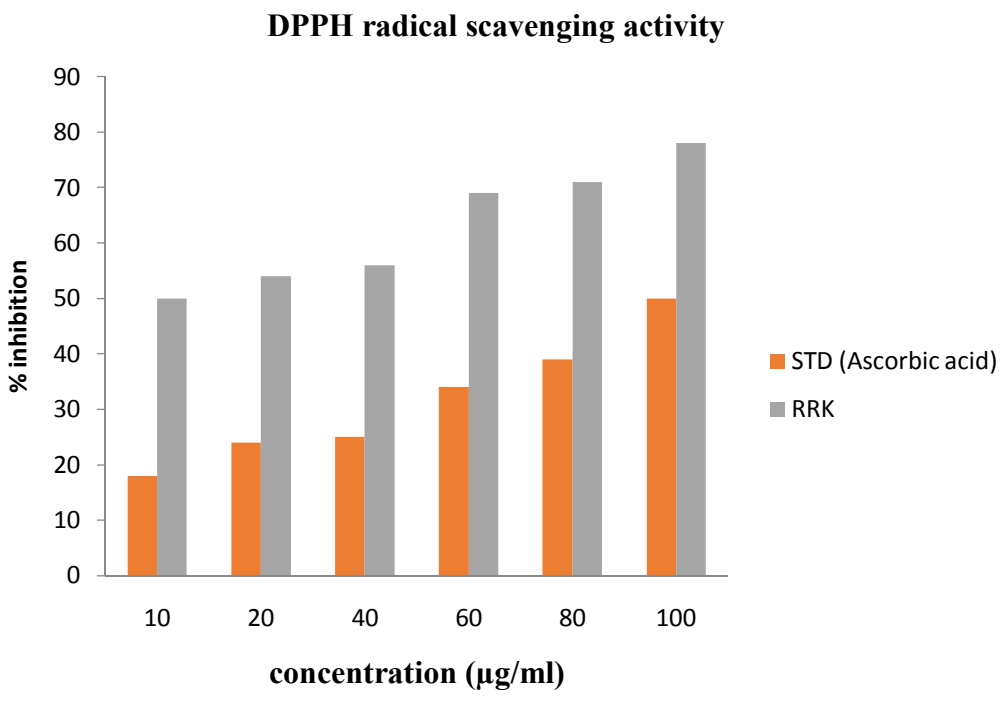
Table : 31 ANTIOXIDANT ACTIVITY OF RASA KARPOORA KULIGAI IN IN VITRO METHODS

DPPH FREE RADICAL SCAVENGING ACTIVITY OF RASA KARPOORA KULIGAI

Si.No	Concentration	Ascorbic acid (Standard)		RKK	
		Absorbance	% inhibition	Absorbance	% inhibition
1	10	1.626	18 %	0.994	50 %
2	20	1.514	24 %	0.825	54 %
3	40	1.492	25 %	0.778	56 %
4	60	1.316	34 %	0.620	69 %
5	80	1.214	39 %	0.585	71 %
6	100	0.988	50 %	0.438	78 %
	Ic 50 values		Ic_{50}= 64.98		Ic_{50} =12.70

Fig : 13 DPPH radical scavenging activity





INTERPRETATION

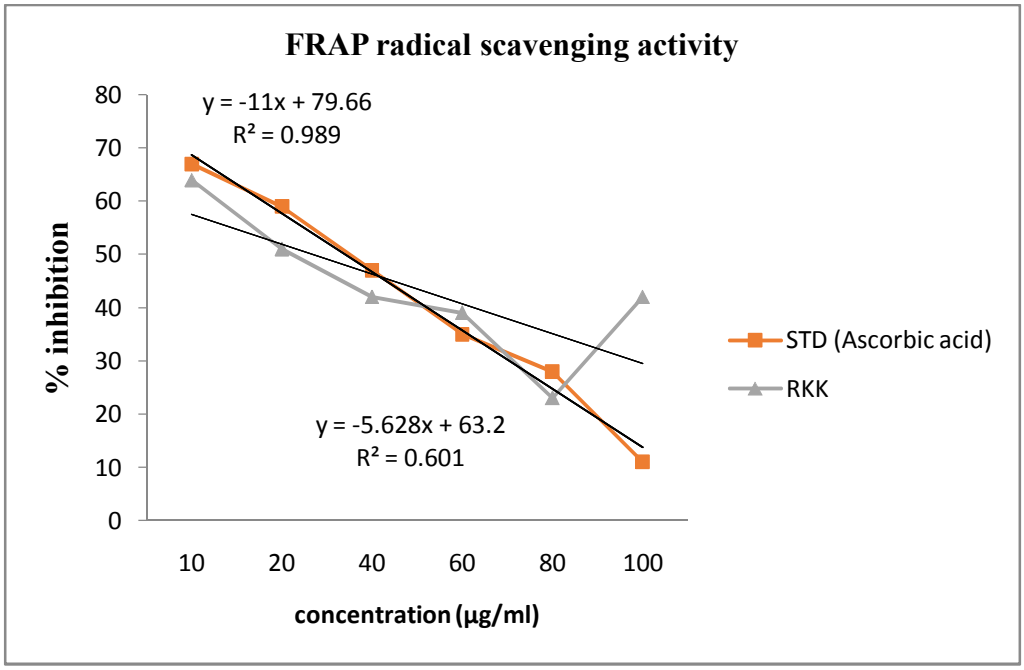
Antioxidants are slow down the oxidative damage of our body. Antioxidants act as a freeradical scavengers. Preventing and repairing damages. Health problems such as Heart diseases, cancer and degenerative disorders are all exacerbated by oxidative damage. TheAntioxidant activity of the drug ***RasakarpooraKuligai (RKK)*** was tested by DPPH assay. The results showed that there was a concentration dependent Antioxidant activity of crude extract of ***RasakarpooraKuligai (RKK)***. At the concentration increased from 10 to 100 µg/ml, percentage of inhibition increased from 50 % to 78 %. At a concentration of 100 µg/mlthere was an increased percentage of inhibition (78 %) in scavenging the free radicals (DPPH). The IC50 value was obtained at 12.70 µg/ml. It showed that ***RasakarpooraKuligai (RKK)*** is having significant anti oxidant activity

Table : 32 FRAP RADICAL SCAVENGING ACTIVITY ON RASA KARPOORA KULIGAI

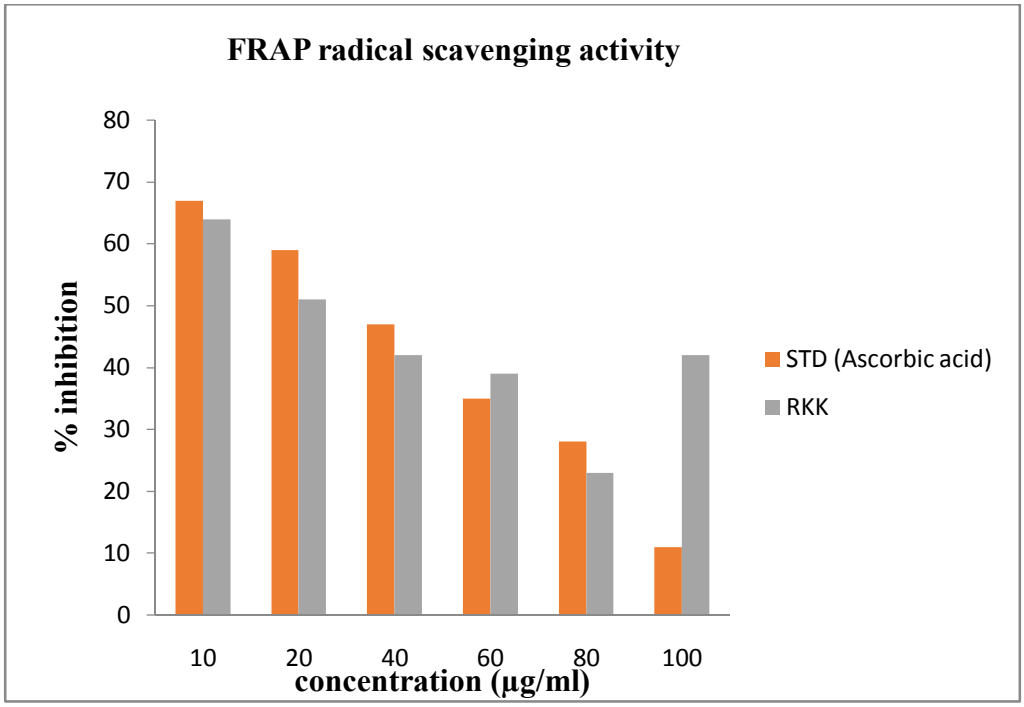
TABLE NO:

Si.No	Concentration	Ascorbic acid (Standard)		RKK	
		Absorbance	**% inhibition**	**Absorbance**	**% inhibition**
1	10	0.286	67 %	0.254	64 %
2	20	0.354	59 %	0.343	51 %
3	40	0.462	47 %	0.470	42 %
4	60	0.566	35 %	0.588	39 %
5	80	0.622	28 %	0.771	23 %
6	100	0.768	11 %	0.982	42 %
	Ic 50 values		Ic_{50}= 26.97		Ic_{50} =23.45

Fig : 14 Percentage of inhibition at different concentration



Percentage of inhibition at different concentration



INTERPRETATION

Many chronic diseases are associated with increased oxidative stress caused by an imbalance between free-radical production and the antioxidant level. Antioxidants, which are abundant in Siddha medicines, they are free-radical scavengers that either reduce the formation of or neutralize free radicals.

The extracts were screened for their potential antioxidant activities using Ferric reducing antioxidant power (FRAP) The DPPH radical scavenging activities were well proved and correlated with the ferric reducing antioxidant capacity of the extracts. Interestingly, The Test drug showed the highest hydroxyl radical scavenging activity of 64% at concentration of 10µg/ml extract. The results showed that there was a concentration dependent Antioxidant activity of crude extract of ***RasakarpooraKuligai (RKK)***. At the concentration increased from 10 to 100 µg/ml,percentage of inhibition increased from 42 % to 64 %. At a concentration of 10 µg/mlthere was an increased percentage of inhibition in scavenging the free radicals (64%). The IC50 value was obtained at 23.75 µg/ml. It showed that ***RasakarpooraKuligai (RKK)*** is having significant **FRAP** radical scavenginganti oxidant activity.

TABLE No.33

ANTI-INFLAMMATORY ACTIVITY OF SIDDHA FORMULATION RKK

Treatment	Dose (mg/kg)	Paw volume(ml) as measured by mercury displacement at 6 hour	Percentage inhibition of paw edema
Group I Normal saline	10ml/kg orally	5.20±0.96	-
Group II Std	10mg/kg I.P.Diclofenac sodium	1.70±0.40	67.30%*a
Group III RKK	200mg/kg.Orally.	2.06±0.48	60.38%*a
Group IV RKK	400mg/kg.Orally.	1.90±0.52	63.46%*a

* Data are expressed as Mean ± S.E.M.

*Data were analyzed by one way ANOVA followed by Newman's keul's multiple range tests, to determine the significance of the difference between the control group and rats treated with the test compounds.

*a Values were significantly different from normal control at P< 0.01.

INTERPRETATION

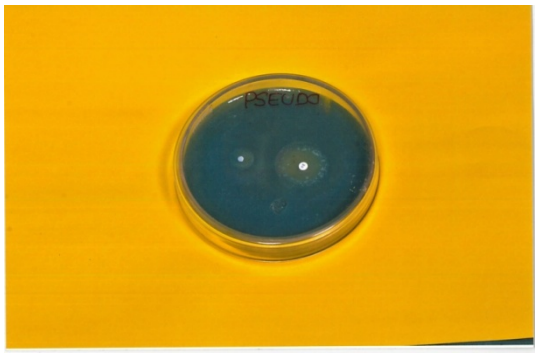
Anti- inflammatory activity

Both doses of siddha formulation RKK at 200mg/kg and 400mg/kg were tested for their Anti- inflammatory activity by using carrageenan Induced rat paw edema method and the results are tabulated in table no 1. The results reveals that both doses of siddha formulation at 200mg/kg and 400mg/kg doses possesses significant Anti- inflammatory activity when compared to control group at p<0.01.

ANTI – MICROBIAL ACTIVITY

Table -34

S.No	Microorganisms	Susceptibility	Zone of Inhibition (mm)	
			Control 100 µgm	Sample
1.	*Escherichia coli*	Sensitive	19	17
2.	Klebsiella pneumonia	Moderately sensitive	14	13
3.	Staphylococcus aureus	Sensitive	16	14
4.	Streptococcus mutans	Moderately sensitive	12	12
5.	Pseudomonas.aerugina	Moderately sensitive	16	9



PSEUDOMONAS

E-COLI

STREPTOCOCCUS

STAPHYLOCOCCUS

KLEBSELLA

Fig : 15

INTERPRETATION:

It was observed that anti microbial studies of showed that it is sensitive Rasa karpoora kuligai against *Escherichia coli,*Staphylococcus aureus and moderately sensitive against Streptococcus mutans,pseudomona aerugina when compared to the standard drug (Amikacin) which was evident from the zone inhibition.The herbo mineral drug DDC showed inhibition of the growth of the micro organism at 100mg/ml concentration for the organism.Our result confirmed the traditional use of RKK has Anti microbial activity.

7.SUMMARY

The test drug RASA KARPOORA KULIGAI is selected from the text, **GUNAPADAM THATHU JEEVAM** for the evaluation of safety, efficacy and therapeutic potency in CERVICAL CANCER *(yoni putru)*.

Aim of the dissertation is to rule out the anti cancer, anti-oxidant, anti-inflammatory properties of the test drug Rasa karpoora kuligai by universal accepted scientific methods.

The review of the literatures and lateral research reveals pepper, garlic, betel leaf, mother milk that, are having anti cancer, anti-oxidant, anti-inflammatory activities .

The test drug was prepared properly by the given procedure all the ingredients were identified and authenticated by the experts

The preparation of trial drug was standardized primarily by physicochemical and biochemical analysis

The physicochemical analysis the drug shows brown in colour with pleasant odour and bitter mixed light pungent in taste.

As per the siddha literature diseases are caused due to changes in mukkutram, in cancer disease the kapam is affected; to treat kapha the drug must be in suitable taste that lower the effect of kapam.

In physiochemical analysis the total Ash value of test drug is 7.3% which shows the total inorganic content (ammonium, potassium, calcium, chloride, iron) present in the drug. These contents are having important role in physiological functions of the body.

Biochemical analysis shows the presence of calcium, sulphate, chloride, starch, phosphate, ferrous iron these compounds are protect the body from the risk of cancer.

In instrumental analysis the ICP-OES result shows the toxic heavy metals such as As, Hg, Cd, Pb, Al, Cu, Ni are in Below Detection Limit(BDL) . The main ingredient of the drug is mercuric II chloride, but the final product Rkk shows below detection limit of the mercury. It is evident that the effectiveness of ***SUDDHI*** *(purification)* of siddha medicine has been proved by the modern scientific way.

The FTIR instrumental analysis shows the presence of phenols, alcohol, amines, aliphatic amines, carboxylic acids, aromatic and alkyl halides groups. From

these phenol compounds are responsible for the anti-oxidant activity of the test drug and other remaining compounds are responsible for the anti microbial and anti inflammatory activity.

SEM analysis of the ***Rasa karpoora kuligai*** shows that the uniform distribution of particles presents in the entire field. Most of the particles present in the sample is nano size and near nano size, average particle size is 4.64µm - 7.51µm which increase the efficacy and bio availability of the test drug. So, very minimal quantity of the medicine is enough to treat the disease.

The acute toxicity study shows that ***Rasa karpoora kuligai*** did not produce any toxic effect at dose of 5mg/kg, 50mg/kg , 300mg/kg, 1000mg/kg and 1750 mg/kg to rats. So No-Observed-Adverse-Effect-Level (NOAEL) of ***Rasa karpoora kuligai*** is 1750 mg/kg

In sub acute toxicity study test drug Rasa *karpoora kuligai* can be considered safe, as it did not cause either any lethality or adverse changes with general behaviour of rats and also there were no observable detrimental effects **(200 to 600 mg/kg body weight)**over a period of 28 days. Our results have demonstrated that the ***Rasa karpoora kuligai*** is relatively safe when administered orally in rats

In the anti cancer study the Cytotoxicty properties of *rasa karpoora kuligai* against hela cell line the percentage growth inhibition was found to be increasing with different concentration of test compound.***Rasakarpoorakuligai*** effect on HeLa cell line at the concentration increased from 62.5 to 1000µg/ml, percentage of inhibition increased from 6.32 % to 94.02% and the IC50 value on HeLa cell line was 164.77µg/ml. Highest percentage of inhibition(94.02%)obtained at the concentration of 1000µg/ml. The results suggested that the ***Rasakarpoorakuligai*** significantly inhibited the proliferation of human cervical cancer HeLa cells.

From the results of DPPH Assay, FRAP radical scavenging methods, it is found that displayed significant antioxidant activity which might be due to its chemical constituent. This suggests that it is useful to prevent the oxidative stress induced damage seen in cancer .Thus it may act as prophylactic as well as curative drug in treating cancer.

In pharmacological study the anti inflammatory activity of Rasa karpoora kuligai results reveals that both doses of RKK at 200mg/kg and 400mg/kg doses possesses significant Anti- inflammatory activity when compared to control group.

Antimicrobial studies show, antimicrobial activity against the E.coli, Pseudomonas aeruginesae, Klebisella pneumonia, streptococcus and stapylo coccus which compared to standard drug(Amikacin). The test drug inhibits the growth of the micro organisms effectively. This is mainly due to the phytochemical constituents present in the Rkk.

It has been summarized that Rasa karpoora kuligai is very effective in treating cervical cancer(yoni putru) without causing any adverse effects.

8. CONCLUSION

Management of cancer with a holistic approach, devoid of any side effects is now the major challenge to the medical system. This work highlights Rasa karpoora kuligai as novel anti cancer agent which provide a basis for the traditional use of it and proves that it could provide a cost effective and holistic remedy, without any side effects. From the literature review, physiochemical, pharmacological, microbiological, phytochemical, Instrumental analysis support the traditional use of *Rasa karpoora kuligai* in *Siddha* system of medicine for the treatment of cervical cancer (*yoni putru*).

9. FUTURE SCOPE

Pre clinical evaluation of the drug ***Rasa karpoora kuligai*** has been done by physio chemical, biochemical, Instrumental, pharmacological, toxicological, antimicrobial procedures.In future,the drug has to be validated by further studies and should be used for patients.

The active principle which is responsible for the activity of ***Rasa karpoora kuligai*** has to be find out, through modern scientific analysis. Having made up of nano particles, holds extra promise for the treatment of cancer.

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